# THE AMERICAN JOURNAL OF PHYSIOLOGY

VOLUME LXVI

BALTIMORE, MD.
1923

SEP 12 1923

66-67


# THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY


## CONTENTS


| | PAGE |
|---|---|
| The Influence of Diet on the Alkaline Reserve and Insulin Hypoglycemia in Rabbits. *Irvine H. Page* | 1 |
| On the Chemical Regulation of Respiration. I. The Regulation of Respiration with Special Reference to the Metabolism of the Respiratory Center and the Coördination of the Dual Function of Hemoglobin. *Robert Gesell* | 5 |
| Further Experiments on the Sensitization to Heat Due to Exposure of Short Wave Lengths. Influence of Ozone. *H. S. Forbes and G. A. Daland* | 50 |
| New Experiments on the Sensitization of Protoplasm to Heat by Exposure to Light of Short Wave-Length. *W. T. Bovie and G. A. Daland* | 55 |
| Studies on the Physiology of Sleep. I. The Effects of Prolonged Sleeplessness on Man. *Nathaniel Kleitman* | 67 |
| The Calorigenic Action of Adrenalin Chlorid. *Walter M. Boothby and Irene Sandiford* | 93 |
| Phase Reversal in Emulsions and Protoplasm. *William Seifriz* | 124 |
| The Effect of Gastrin and Histamine on the Movements of the Stomach and Intestine During Hunger. *A. C. Ivy and D. A. Vloedman* | 140 |
| Seasonal Changes in the Gases and Sugar of the Blood and the Nitrogen Distribution in the Blood and Urine of the Alligator. *Aleita Hopping* | 145 |
| Studies in the Physiology of Vitamins. II. Parenteral Administration of Vitamin-B—Mammalian Experiments. *George R. Cowgill* | 164 |
| The Effect of Fatigued Muscle upon Perfused Adrenalin. *R. G. Hoskins and E. P. Durrant* | 176 |
| Studies on the Locomotion of Leucocytes. I. The Normal Rate of Locomotion of Human Neutrophilic Leucocytes in Vitro. *Morton McCutcheon* | 180 |
| Studies on the Locomotion of Leucocytes. II. The Effect of Temperature on the Rate of Locomotion of Human Neutrophilic Leucocytes in Vitro. *Morton McCutcheon* | 185 |
| Some Effects of Various Environmental Temperatures upon the Blood of Dogs. *Frederick B. Flinn and Ernest L. Scott* | 191 |
| On Absorption from the Cloaca in Birds. *N. C. Sharpe* | 209 |



Vol. LXVI—No. 1

*Issued September 1, 1923*

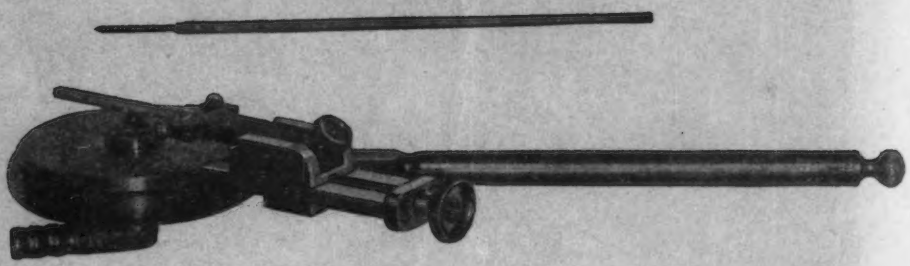
BALTIMORE, U. S. A.

1923

tered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917. Authorized on July 5, 1918

*Made in United States of America*

# AIR TAMBOUR



THE instrument pictured above is very sensitive to changes in air pressure, but is so well made that it will give satisfactory service for years. The extent of the excursion of the writing point can be increased or decreased by turning a milled screw head. It can be adjusted to any position in the horizontal plane and its level is adjusted by means of a small screw. The entire surface of the rubber, except for a small central area, is protected by a metal diaphragm. The rubber is laid on the air capsule, a brass ring is placed over it, and then the air capsule is screwed against the metal diaphragm. In this way tying or stretching of the rubber is eliminated.

WHEN not in use, a small cap is screwed over the central area, and if a cork is pushed into the air-tube the capsule is rendered air-tight and the rubber dam will last indefinitely.

**PRICE $14.50**

***Catalogue on Request***

## JOSEPH BECKER

**437 WEST 59TH STREET** **NEW YORK**

# CONTENTS

## No. 1. September, 1923


The Influence of Diet on the Alkaline Reserve and Insulin Hypoglycemia in Rabbits. *Irvine H. Page* ..... 1

On the Chemical Regulation of Respiration. I. The Regulation of Respiration with Special Reference to the Metabolism of the Respiratory Center and the Coördination of the Dual Function of Hemoglobin. *Robert Gesell* ..... 5

Further Experiments on the Sensitization to Heat Due to Exposure of Short Wave-Lengths. Influence of Ozone. *H. S. Forbes and G. A. Daland* ..... 50

New Experiments on the Sensitization of Protoplasm to Heat by Exposure to Light of Short Wave-Length. *W. T. Bovie and G. A. Daland* ..... 55

Studies on the Physiology of Sleep. I. The Effects of Prolonged Sleeplessness on Man. *Nathaniel Kleitman* ..... 67

The Calorigenic Action of Adrenalin Chlorid. *Walter M. Boothby and Irene Sandiford* ..... 93

Phase Reversal in Emulsions and Protoplasm. *William Seifriz* ..... 124

The Effect of Gastrin and Histamine on the Movements of the Stomach and Intestine During Hunger. *A. C. Ivy and D. A. Vloedman* ..... 140

Seasonal Changes in the Gases and Sugar of the Blood and the Nitrogen Distribution in the Blood and Urine of the Alligator. *Aleita Hopping* ..... 145

Studies in the Physiology of Vitamins. II. Parenteral Administration of Vitamin-B—Mammalian Experiments. *George R. Cowgill* ..... 164

The Effect of Fatigued Muscle upon Perfused Adrenalin. *R. G. Hoskins and E. P. Durrant* ..... 176

Studies on the Locomotion of Leucocytes. I. The Normal Rate of Locomotion of Human Neutrophilic Leucocytes in Vitro. *Morton McCutcheon* ..... 180

Studies on the Locomotion of Leucocytes. II. The Effect of Temperature on the Rate of Locomotion of Human Neutrophilic Leucocytes in Vitro. *Morton McCutcheon* ..... 185

Some Effects of Various Environmental Temperatures upon the Blood of Dogs. *Frederick B. Flinn and Ernest L. Scott* ..... 191

On Absorption from the Cloaca in Birds. *N. C. Sharpe* ..... 209


## No. 2. October, 1923


Biological Food Tests. IV. The Protein and the Vitamin-A Content of the English Walnut. *Helen L. Mignon* ..... 215

On the Sources of Heat in Muscle Contraction. *Charles D. Snyder* ..... 232

The Average Epinephrin Output in Cats and Dogs. *G. N. Stewart and J. M. Rogoff* ..... 235

On the Function of the Cerebral Ventricles. I. Demonstration of Small Changes in the Capacity of the Lateral Ventricles of the Brain. *K. J. Austmann and V. H. K. Moorhouse* ..... 267
The Rôle of Anoxemia in the Causation of Tetany During Hyperpnea. *Samuel B. Grant* ..... 274
The Relations Between Lesions of the Brain Stem, Water Elimination and Body Temperature. (Preliminary Report.) *Fred T. Rogers* ..... 284
The Gill Movements in One of the Perennibranchiate Urodela (Necturus maculatus) and Their Relation to the Central Nervous System. *G. N. Stewart* ..... 288
The Quantitative Determination of Trypsin and Lipase in Gastric Contents. *C. W. Lueders and Olaf Bergeim* ..... 297
The Effect of Asphyxia Upon the Adrenal Output as Demonstrated by Variations in the Temperature of the Brain. *George W. Crile, Amy F. Rowland and S. W. Wallace* ..... 304
Studies on the Physiology of Reproduction in Birds. XIII. Asphyxial Death of Embryos in Eggs Abnormally Retained in the Oviduct. *Oscar Riddle* ..... 309
Studies on the Physiology of Reproduction in Birds. XIV. Suprarenal Hypertrophy Coincident with Ovulation. *Oscar Riddle* ..... 322
Studies on the Physiology of Reproduction in Birds. XV. Increased Blood Sugar Coincident with Ovulation in Various Kinds of Pigeons. *Oscar Riddle and Hannah Elizabeth Honeywell* ..... 340
The Mineral Metabolism of the Lactating and Dry Goat. *Chas. H. Hunt, A. R. Winter, J. A. Schultz and R. C. Miller* ..... 349
The Effect of Diuresis on Excretion of Uric Acid. *J. Lucien Morris and H. Maynard Rees* ..... 363
The Production of Hypoglycemia in Experimental Derangements of the Liver. *Meyer Bodansky* ..... 375
Studies in Peristaltic Fatigue. *Velyien E. Henderson* ..... 380
Reflex Contractions of an All-or-None Character in the Spinal Cat. *Eugene L. Porter and Victor W. Hart* ..... 391
Further Observations on the Antigenic Effects of Semen. Mechanism of Sterilization of Female Rat from Injections of Spermatozoa. *J. L. McCartney* ..... 404
Adrenal Discharge in the Dog. *Jackson Searles* ..... 408
Further Studies of the Reaction of the Small Intestine to Sodium Bicarbonate. *C. E. King and Jas. G. Church* ..... 414
The Motor Reaction of the Muscularis Mucosae to Some Drugs. *C. E. King and Jas. G. Church* ..... 428
The Effect of Insulin on the Central Nervous System and its Relation to the Pituitary Body. *J. M. D. Olmsted and H. D. Logan* ..... 437

No. 3. November, 1923

The Nature of the Activities of the Human Sweat Glands. *Edward F. Adolph* ..... 445
Studies of the Brain Stem. VII. The Respiratory Exchange and Heat Production after Destruction of the Body Temperature-Regulating Centers of the Thalmus. *F. T. Rogers and R. W. Lackey* ..... 453

A Study of the Rabbit as a Test Animal for Determining the Potency of Insulin Preparations. *H. D. Clough, R. S. Allen and E. W. Root, Jr.* ..... 461
Contributions to our Knowledge of the Autonomic Control of Liver Function. I. The Action of Autonomomimetic Drugs, and of Vagal Stimulation upon Fluid and Sugar Output from the Hepatic Veins of the Terrapin. *C. D. Snyder, H. S. Wells and P. G. Culley* ..... 485
Some Conditions Affecting Catalytic Action of Blood. *Lalia Walling and O. O. Stoland* ..... 503
Experimental Ablation of the Superior Cervical Sympathetic Ganglia of the Rabbit. *Frank P. Underhill and George T. Pack* ..... 519
The Influence of Various Diuretics on the Concentration of the Blood. *Frank P. Underhill and George T. Pack* ..... 520
The Nature of the Delay in the Response to the Second of two Stimuli in Nerve and in the Nerve-Muscle Preparation. *A. Forbes, L. H. Ray and F. R. Griffith, Jr.* ..... 553
Reflex Hyperglycemia: A Study of the Carbohydrate Mobilization Effected by Afferent Crural, Sciatic and Vagus Stimulation. *Fred R. Griffith, Jr.* ..... 618
Further Evidence Regarding the Relationship of the Adrenals to the Amount of Sugar in the Blood. *Fred R. Griffith, Jr.* ..... 659
Index ..... 665

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 66 SEPTEMBER 1, 1923 No. 1

# THE INFLUENCE OF DIET ON THE ALKALINE RESERVE AND INSULIN HYPOGLYCEMIA IN RABBITS

IRVINE H. PAGE

*Contribution from The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana*

Received for publication May 19, 1923

In the course of some experimental work on the resistance of rabbits to insulin hypoglycemia, we have found that rabbits maintained on a diet of oats and hay showed falls in blood sugar proportional to the dosage of insulin in a much more consistent manner than rabbits on cabbage and hay. Sherman and Gettler (1) have shown that cereals such as oats produce a strongly acid ash whereas cabbage burns to a basic ash. McDanell and Underhill (2) concluded that in normal rabbits which maintained their weight, no significant change in blood sugar resulted from change in the acid-base relation of the diet.

The hypoglycemia incident to insulin injection is the result of a number of factors; primarily that of glycogenesis or sugar polymerization and secondarily increased carbohydrate metabolism. It is well known that a fall in alkaline reserve is associated with hyperglycemia, hence if we can introduce into the organism any factor that will tend to produce such a fall we will render the animal more resistant to the production of hypoglycemia. This paradoxical acidosis has been dealt with in another paper.

The present investigation will show that diet is such a factor and that rabbits kept on an acid-forming ration are more resistant to insulin hypoglycemia than those on basic diets. Also that the alkaline reserve is markedly raised on a basic diet whereas it is lowered on an acid maintenance.

*Experimental.* One and one-half to 2-kilogram rabbits were placed on *a*, acid diets of oats and bread, and *b*, basic diets of cabbage, carrots and hay. Alkaline reserves were run employing the method of Van Slyke and Cullen (3), taking care to introduce as little hemorrhage as possible while bleeding the rabbits. The following table gives the results of these determinations:

TABLE 1

*Alkaline reserve*

| RABBIT NUMBER | 1 WEEK | 2 WEEKS | 3 WEEKS | 4 WEEKS | 5 WEEKS |
|---|---|---|---|---|---|
| | | | Acid diet | | |
| 8250 | 39.9 | 48.58 | 40.8 | 64.5 | 54.04 |
| 8221 | 53.12 | 55.88 | 38.9 | 53.2 | 60.66 |
| 8298 | 47.42 | 27.80 | | | |
| 9205 | 56.00 | 45.46 | | | |
| 8254 | 59.78 | 62.56 | 41.94 | 52.2 | 61.70 |
| 4471 | 58.7 | 37.6 | | | |
| 4476 | 52.5 | 41.8 | | | |
| 4470 | 52.07 | 51.08 | | | |
| 4476 | 52.5 | 41.94 | | | |
| 7102 | 38.9 | 48.02 | | | |
| 3465 | 43.3 | 53.80 | | | |
| 7136 | 49.16 | 74.88 | | | |
| 3884 | 62.44 | 63.36 | | | |
| 3462 | 51.06 | 61.40 | | | |
| Average..... | 51.20 | 51.01 | 40.54 | 56.6 | 58.80 |
| | | | Basic diet | | |
| 5881 | 76.8 | | | | |
| 6119 | 57.9 | | | | |
| 6057 | 75.8 | 81.50 | | | |
| 6055 | 84.32 | | | | |
| 6644 | 58.78 | 66.36 | | | |
| 5899 | 73.98 | | | | |
| 6165 | 72.04 | 74.80 | | | |
| 6178 | 64.38 | | | | |
| 6106 | 45.34 | 86.2 | | | |
| 6102 | 81.50 | | | | |
| 6187 | 77.70 | 86.20 | | | |
| 7737 | 60.54 | | | | |
| 6036 | 80.58 | 67.24 | | | |
| 5807 | 84.32 | 81.50 | | | |
| 5826 | 71.10 | | | | |
| 5884 | 75.80 | | | | |
| 5823 | 71.10 | | | | |
| Average..... | 74.39 | 77.40 | | | |

It will be seen that rabbits on a basic diet have a $CO_2$ reserve capacity far in excess of that exhibited by those maintained on an acid diet.

The basic diet employed did not maintain the animals in good health. At the end of 2 weeks a rather severe diarrhea usually set in, entailing loss in weight. Animals losing weight certainly are less resistant to

hypoglycemia than those gaining or at least maintaining their weight. These factors must not be overlooked in any work of this type. The following table presents the average of the results of 30 rabbits on acid and 28 on basic diets.

The rabbits on hay and cabbage averaged initially a higher weight than those on oats but at the end of 12 days averaged somewhat lower. The minimum convulsive dose of insulin was given subcutaneously and blood sugars taken 2 and 4 hours after injection. The initial drop on the basic diet averaged very decidedly lower than those on the acid. This difference was temporary, however.

A factor of some importance at certain seasons of the year is the appearance of disease of the liver known as Coccidium hepatis or "spotty liver." The disease spreads rapidly, generally being contracted from unclean watering troughs. It may be so acute as to involve 80 per cent of the liver. The syndrome in severe cases is a staggering gait, with twitching, slightly convulsive movements. Nystagmus may be present.

TABLE 2

*Effect of diet on sugar regulating mechanism*

| | BASIC ASH DIET | ACID ASH DIET |
|---|---|---|
| Weight in grams after 2 days | 1267 | 1195 |
| Weight in grams after 12 days | 1046 | 1139 |
| Blood sugar 2 hours after insulin (per cent) | 0.027 | 0.054 |
| Blood sugar 4 hours after insulin (per cent) | 0.063 | 0.065 |

Animals in this condition are peculiarly sensitive to the hypoglycemic effects of insulin, hence should be especially guarded against in this type of experiment.

Discussion. The results seem to indicate that the acid- and base-forming properties of diets are of considerable importance in the maintenance of blood sugar levels in the rabbit. The alkaline reserve is certainly influenced by diet, and, since the reserve is an index of acid-base balance in the blood, and since blood sugar regulation is at least partially controlled by this balance, we conclude that diet influences the regulating mechanism of the blood sugar. As previously pointed out, any factor that will aid glycogenolysis—such as acidosis—will tend to render the organism less susceptible to hypoglycemias. Diet is at least one of those factors.

The author wishes to express his sincere appreciation to Mr. W. O. Rader for aid in the experiments, and to Dr. G. H. A. Clowes for his interest throughout the work.

## CONCLUSIONS

1. Acid ash forming diets produce a condition of mild acidosis in rabbits whereas basic ash diets tend toward the production of alkalosis.

2. Acid diets render rabbits more resistant to the hypoglycemic effects of insulin than basic diets by tending to produce a condition of acidosis, thus increasing glycogenolysis and in this way antagonizing the insulin action.

3. Diet in the rabbit is an important factor in the regulation of the blood sugar level.

## BIBLIOGRAPHY

(1) Sherman and Gettler: Journ. Biol. Chem., 1912, xi, 323.
(2) McDanell and Underhill: Journ. Biol. Chem., 1917, xxix, 233.
(3) Van Slyke and Cullen: Journ. Biol. Chem., 1917, xxx, 289.

# ON THE CHEMICAL REGULATION OF RESPIRATION

## I. The Regulation of Respiration with Special Reference to the Metabolism of the Respiratory Center and the Coördination of the Dual Function of Hemoglobin[1]

ROBERT GESELL

*From the Washington University School of Medicine, St. Louis*

Received for publication May 25, 1923

I. Theories on the chemical regulation of respiration. On reviewing some of the abundant literature which has accumulated on the mechanism of the chemical control of respiration, we were impressed with the multiplicity of views arising from a mass of data which is relatively uniform. On the whole, it can be said that interpretations rather than findings are contested. For example, during suspended respiration, two important chemical changes occur in the blood, a decrease in the tension of oxygen and an increase in the tension of carbon dioxide. The relative significance of these two changes in ultimately stimulating respiration, however, is still debated. Furthermore, the exact mode of action of lack of oxygen and excess of carbon dioxide is also contested. There are at least five theories on the mode of action of lack of oxygen and three on the mode of action of carbon dioxide.

Theories on the mode of action of lack of oxygen:

1. Direct action on the respiratory center.

2. Regulation of the excitability of the center to the normal respiratory stimulus.

3. Indirect stimulation of the respiratory center through the formation of lactic acid which, reaching the blood, lowers the alkaline reserve and raises its hydrogen ion concentration.

4. Indirect stimulation of the respiratory center through the formation of lactic acid within the center itself.

5. Indirect stimulation of the respiratory center by the formation of easily oxidizable substances, other than acids, which during lack of

[1] Preliminary reports:
Gesell: This Journal, 1923, lxiii, 393.
Gesell, Foote and Capp: Proc. Soc. Exper. Biol. and Med., 1922, xx, 174.
Gesell: Proc. Soc. Exper. Biol. and Med., 1923, xx, 345.

oxygen reach the blood and pass the lungs in excessive amounts and excite the center.

Theories on the mode of action of carbon dioxide:

1. Stimulation by virtue of its effects on the hydrogen ion concentration of the blood.

2. Stimulation by virtue of its effects on the hydrogen ion cencentration of the cell after having entered the cell.

3. Stimulation by virtue of a specific effect other than changes in the hydrogen ion concentration.

When Haldane (1) states in his recent book that the acidity of the arterial blood is regulated to a degree defying detection by our present methods he, with others, emphasizes the close correspondence between respiratory activity and the composition of the arterial blood. But the correspondence between the composition of arterial blood and pulmonary ventilation appears to be in need of analysis. For example, if the hydrogen ion concentration of the arterial blood determines ventilation, we must not only explain the hyperpnea of acidemia, but also the hyperpnea of alkalemia which we know exists during anoxemia (2), (3). Likewise, if the specificity of carbon dioxide is an important factor in the stimulation of the respiratory center, we must not only explain the hyperpnea occurring with alkaline blood of high carbon dioxide tension as demonstrated by Scott (4), but also the hyperpnea associated with alkaline blood of low carbon dioxide tension occurring in anoxemia. And finally, if lack of oxygen excites the respiratory center, what is the mechanism of hyperpnea associated with excess of oxygen?

So far as we are aware, there is no satisfactory theory which satisfies all of these conditions. Haldane (5) and Haggard and Henderson (2) have recently recognized the insufficiency of the present theories in proposing the formation of an unknown substance in the body during anoxemia which, passing the lungs in greater abundance than under normal circumstances, increases respiration. This substance, according to Haggard and Henderson, is not an acid and is designated as Respiratory X.

Despite the accumulating data tending to minimize the significance of the hydrogen ion, the introduction of a new hypothetical substance at this point appears unnecessary, for the possibilities of an explanation of pu'monary ventilation on the basis of the hydrogen ion have not been exhausted.

A lack of correspondence between pulmonary ventilation and the hydrogen ion concentration of the blood need not necessarily exclude the hydrogen ion as the regulator of respiration. Our experiments on the relation of blood volume to tissue nutrition have inclined us to

view changes in the metabolism of the respiratory center itself with its consequent changes in acidity as a factor in the chemical control of respiration. This view demands a correlation of respiration and the acidity of the center rather than of the acidity of the arterial blood. Since the acidity of the center and of the blood may vary in opposite directions we believe our experiments supply a basis for unifying long existent data and views on the mechanism controlling pulmonary ventilation and the hydrogen ion concentration of the tissues. Our theory may be outlined as follows.

Changes in the hydrogen ion concentration of the respiratory center, rather than of the blood, constitute the determining factor in respiratory control.

Since the supply of oxygen determines the absolute and relative amounts of lactic acid and carbon dioxide formed in living tissues and since it controls the efficiency of transport and elimination of acid, it constitutes the normal and indirect regulator of pulmonary ventilation.

The effects of lactic and carbonic acid are additive. They are exerted indirectly by way of the blood and, more directly, through their formation in the respiratory center itself.

Diminished oxidation in the respiratory center leads to an accumulation there of the relatively poorly diffusible lactic acid in a relatively poorly buffered cytoplasm and lymph, no longer fully protected by the potential alkalinity of the blood, the dual function of hemoglobin being disturbed by the diminution in the reduction of hemoglobin and the liberation of alkali as it passes through the center.

By virtue of its own metabolism and its extreme sensitivity to minute changes in its own hydrogen ion concentration the respiratory center is sensitive to minute changes in its own oxidations and, therefore, to changes in the tension of oxygen in the arterial blood.

The capacity of the center to respond to changes in the arterial carbon dioxide tension consequent on fluctuations in the general metabolism, however, must also be a factor.

Insofar as the normal respiratory activity is a combined effect of reflex stimulation and of physico-chemical changes within the center, we suggest that the coördination of these two factors is dependent on the augmentation (6) of respiratory reflexes by increased acidity.

Though pulmonary ventilation and acidity are closely coupled in our theory, we fully realize that an invariable accompaniment of a phenomenon need not necessarily be its cause; yet the extreme sensitivity of the respiratory center to the hydrogen ion warrants a study of the causal relation of changes in the hydrogen ion concentration to changes in pulmonary ventilation.

II. Specificity of carbon dioxide as a regulator of respiration. Winterstein (7) perfused young rabbits through the aorta and found that oxygenated Ringer's solution free of carbon dioxide produced apnea; that subsequent perfusion with oxygenated solution plus carbon dioxide restored respiration; that apnea produced by a lack of carbon dioxide was not interrupted by substituting Ringer's solution free of oxygen; that the addition of other acids, such as hydrochloric acid, produced respiration. Winterstein concluded that neither the lack of oxygen nor the tension of carbon dioxide in themselves, but rather the hydrogen ion concentration of the blood, singly and alone, serves as the chemical regulator of respiration.

Laquer and Verzar (8) confirm the findings of Winterstein, but maintain that the general mechanism of stimulation is not explainable on the basis of the hydrogen ion concentration of the perfusate. The general similarity of action of the various acids is due to the increased liberation of $CO_2$, $H_2CO_3$ and $HCO_3$ in the tissues. They state that carbon dioxide at all events has a specific exciting effect which exerts itself in neutral and even alkaline reaction.

By altering the alveolar carbon dioxide tension through diet, Hasselbalch (9) concluded that the hydrogen ion concentration of the blood rather than the carbon dioxide tension was the important factor in the regulation of respiration.

The more recent experiments of Hooker, Wilson and Connett (10) on the perfused mammalian medulla, however, point definitely to the significance of the tension of carbon dioxide in the blood. They compared the effects of two samples of blood of different carbon dioxide tensions but of the same hydrogen ion concentration, and found that the blood possessing the higher carbon dioxide tension elicited the greater respiration. As they assumed that an acid equilibrium was established in both instances between the respiratory center and the blood, they concluded that carbonic acid exerts a specific action upon the respiratory center.

Scott (4) also established a lack of parallelism between the hydrogen ion concentration of the blood and pulmonary ventilation. With similar administration of carbon dioxide before and after intravenous injection of sodium bicarbonate, approximately equal respiratory response was elicited even though a lower hydrogen ion concentration obtained in the latter case. Scott believes that the undissociated $CO_2$ acts as a specific respiratory hormone and consequently the physiological effects of carbon dioxide cannot be attributed solely to its acid properties.

Jacobs (11), (12) has offered another suggestion. He demonstrated that an organism placed in an alkaline sodium bicarbonate buffer solu-

tion of high carbon dioxide tension turns acid, and believes that such results may explain the so-called specific action of carbon dioxide on the respiratory center.

Collip (13), on the other hand, calls attention to the increased pulmonary ventilation which may follow the intravenous injection of sodium bicarbonate. Since such injection decreases the hydrogen ion concentration of the blood, he concludes that the respiratory center has a specific sensitivity to the $HCO_3$ ion. Dale and Evans (14) also find that the injection of sodium bicarbonate may decrease the duration of apnea produced by forced respiration, but their conclusions are different.

Dale and Evans (14) studied the fall in blood pressure elicited by excessive pulmonary ventilation and found that such ventilation invariably decreased the hydrogen ion concentration of the arterial blood which was not corrected as the blood passed through the tissues. They found that pulmonary ventilation elicited the same fall in blood pressure after the injection of acid when the blood was well on the acid side of normal and furthermore, that a reduction in the acidity of the blood equal to that produced by ventilation, when accomplished by the injection of sodium bicarbonate, was ineffective. They state that "the results are in line with a number of observations which have appeared during recent years, and which seem to indicate that free carbon dioxide as such and apart from the increase of H ions produced by its addition to the blood, has a specific stimulant action on the respiratory center." They further state that, "the meaning of this specific effect of $CO_2$ as distinguished from acidity is by no means clear; that the fall of arterial pressure, which has been the subject of this investigation, is the result of acapnia in the original sense given to the term by Mosso, as accepted by Henderson in his earlier papers dealing with excessive ventilation, and not of 'alkalosis' as more recent speculations have suggested."

Obviously if the striking effect of pulmonary ventilation upon blood pressure noted by Dale and Evans and if the apnea following forced respiration are due to the removal of a specific effect of carbon dioxide, rather than to a change in alkalinity, and if the hyperpnea produced by the injection of sodium bicarbonate is attributable to a specific action of the $HCO_3$ ion, the theory we have presented cannot stand.

The problem of the chemical control of respiration is an extremely complex one; a number of fundamental points bearing on the intimate mechanism of the rhythm of the respiratory center are still obscure. As regards the effect of acid on the center, we know neither its intimate nature nor its site of action. Bayliss (15) calls attention to experiments showing chemical influence on living cells through action

on the membrane itself. Sodium hydroxide, though not entering the cells, increases oxidations in the developing sea urchin egg and, likewise, acids accelerate the movements of medusae, although no change of dye indicator within the cells occurs. On the other hand, Jacobs (11), (12) suggests that the acidity of the interior of the cells of the respiratory center may determine respiratory activity.

Whether acid exerts its effect in the respiratory center at the surface of the nerve element, for example, at the synapse or in the interior, or whether increased acidity within the cell may still produce its effects on the membrane by working from within, are points which must ultimately be solved.

We know from Scott's (4) experiments that increased respiration occurs when the blood is on the alkaline side of normal. If the lymph up to the very cell membrane of the nerve elements is also alkaline and the hydrogen ion is the factor controlling respiration, acidity presumably exerts its effects within the cell or on the membrane from within. On the other hand, if the buffers of the lymph are less effective than those in the blood, fluctuation in the amount of carbon dioxide in this lymph would produce changes in acidity at the outer surface of the nerve elements out of proportion to those occurring in the blood. Care must, therefore, be exercised in translating the values of the pH of the arterial blood to the respiratory center. We know, for example, (16) that sodium bicarbonate diffuses very slowly from the blood into the cerebro-spinal fluid and that the hydrogen ion concentration of the latter is higher than that of the circulating blood.

The question of the amount and nature of the buffers[2] of the blood, the lymph and the tissues, (including the respiratory center), the relative permeability of the nerve elements and the tissues as a whole to the constituents of the buffer mixture of the body, the rate of diffusion of these constituents, the effect of sodium bicarbonate on the amounts of the various forms of carbon dioxide in the tissues and tissue fluids, the effect of salts on the rate of diffusion of acid and base, the adsorption of H and OH ions at the cell membrane, the effect of salts on this adsorption (17), etc., all bear directly on the problem of the chemical control of respiration.

Jacobs (12) emphasizes the importance of the selective solubility of carbon dioxide in the cell membrane in relation to the acidity of the interior of the living cell and has tested his views on a model cell with a lipoid solvent membrane possessing greater solubility for carbon dioxide than for sodium bicarbonate. On placing this cell filled with a weak bicarbonate buffer solution in a relatively alkaline buffer solu-

[2] We have considered only changes in the carbonate buffer system in relation to the chemical regulation of respiration.

tion, but with greater concentration of sodium bicarbonate and higher tension of carbon dioxide, he found that the contents of the cell turn acid.

We have recently (18) demonstrated a striking disturbance in the hydrogen ion equilibrium of a solution without the use of a membrane. We prepared a relatively weak and a relatively strong solution of sodium bicarbonate and with the aid of phenol red and carbon dioxide raised the hydrogen ion concentration of each to the same point. The two solutions were carefully stratified in a cylindrical separatory funnel. The denser solution being below, none of the constituents of the solutions can mix except by the process of diffusion. In a few moments a light colored acid ring appears at the interface. This ring gradually increases in width and simultaneously a dark alkaline ring develops below. The formation of colors widens and slowly ascends.

The explanation of the phenomena is, of course, obvious. Any substance in solution tends to diffuse from a point of high concentration to a point of lower concentration. Sodium bicarbonate and carbon dioxide will, therefore, tend to diffuse upwards, but as a result of the higher rate of diffusion of carbon dioxide the disproportionate entrance of carbon dioxide in the upper solution increases the $\frac{H_2CO_3}{NaHCO_3}$ ratio and similarly the disproportionate departure of carbon dioxide from the lower solution decreases the ratio with a consequent display of colors. Whether the lower solution has a lower acidity than the upper naturally has no influence on the general results, provided the carbon dioxide concentration is greater below than above.

Though the results are those which might be expected, yet they are very impressive. Considering the upper solution of our diffusion experiment as the respiratory center and the lower as the blood, in the formation of a ring of colors, over a centimeter wide, representing a disturbance of the hydrogen ion equilibrium from approximately pH 7 to pH 8 we have a striking demonstration of the possible effects of carbon dioxide independent of changes in the hydrogen ion concentration of the blood. And in the separatory funnel these disturbances in the hydrogen ion equilibrium occur without the aid of a membrane, that is, without a differential barrier to the migration of the numerator and denominator of the buffer mixture other than the differential rates of diffusion. An additional differential barrier, for example, the impermeability of the membrane to the sodium bicarbonate, should of course accentuate the exceedingly rapid process exemplified by the simple diffusion experiment. Undoubtedly, such hindrance occurs between the blood and the interior of the nerve cell. Whether it occurs between the blood and lymph of the respiratory center or be-

tween the lymph and the respiratory center is an important question which should be solved.

Some experiments of Fletcher (19) bear on the nature of this differential barrier between the cell and its surrounding media. He found that the rate of escape of carbon dioxide from living muscle, dead muscle, coagulated egg-albumen and water, all followed the same logarithmic curve indicating that the selective solubility of carbon dioxide in the cell membrane is not a significant factor in the hydrogen ion equilibrium of the tissues. The important factor is probably the relative impermeability of cell membranes to the metallic cation.

The degree of this impermeability may be an ultimate factor explaining various anomalous effects of the injection of sodium bicarbonate and bicarbonate buffer mixtures. How, for example, is it possible to explain the slowing of respiration frequently produced by the injection of sodium bicarbonate, if the acidity of the interior of the cell is the controlling factor and the cell membrane is absolutely impermeable to the base? Obviously, sodium bicarbonate should have no retarding effect, unless it enters the cell and reduces the $\frac{H_2CO_3}{NaHCO_3}$ ratio. This possibility should be carefully considered.

On the other hand, it is not improbable that the carbon dioxide, which is formed within the cells of the respiratory center, stimulates the center by affecting the membrane after leaving the cell. Under such circumstances the carbon dioxide, which is passing into the lymph or intersynaptic substance, tends to form an acid pellicle about the cell, the greatest hydrogen ion concentration occurring nearest the cell membrane. Opposing this process is the steady flow of lymph and the diffusion of base from the blood to the site of stimulation which tends to decrease the $\frac{H_2CO_3}{NaHCO_3}$ ratio and slow respiration. This counter-process is presumably increased by the injection of sodium bicarbonate.

But in relation with these vague but necessary considerations, how are we to explain the not infrequent hyperpnea associated with the injection of sodium bicarbonate? Howell many years ago called attention to the stimulating effect of sodium bicarbonate on circulation and respiration. We have frequently obtained similar results and recently Collip (13) has offered as an explanation of this anomalous phenomenon the stimulating effect of the $HCO_3$ ion on the respiratory center.

Another possible explanation has occurred to us. It is known that when carbon dioxide is dissolved in water it is present in three different forms—dissolved $CO_2$, undissociated $H_2CO_3$ and dissociated $H_2CO_3$. Thus $CO_2 \rightleftharpoons H_2CO_3 \rightleftharpoons H^{\cdot} CO_3'$. Addition of sodium bicarbonate pushes

the reaction to the left increasing both the dissolved $CO_2$ and the undissociated $H_2CO_3$. If we accept Loeb's statements (20), (21) that weak undissociated acids diffuse very freely through cell membranes, and Jacobs' view that "The general mode of action of $CO_2$ may be pictured as follows. In the medium surrounding the cell there are $H'CO_3'$ ions and $CO_3$ ions and $CO_2$ and $H_2CO_3$ molecules. The ions as such do not penetrate to any appreciable extent though the H ion at least acting at the cell membrane may have a stimulating effect,"—it would follow that the intravenous injection of sodium bicarbonate increases the freely diffusible forms of carbon dioxide at the expense of the poorly diffusible ions thus increasing the acid effects of the blood on the interior of the cells, even though the sum total of the dissolved carbon dioxide in its various forms is not increased and the hydrogen ion concentration of the blood is actually decreased. This effect alone, neglecting the formation of carbon dioxide in the tissues, would tend to produce diffusion of carbon dioxide against a positive gradient; that is, the injection of sodium bicarbonate should theoretically produce acidosis along with alkalemia[3] by virtue of the greater accumulation of carbon dioxide in the tissues. The hyperpnea (13), the rise in blood pressure (14) and the shortening of artificially produced apnea (14) following the injection of sodium bicarbonate suggest that a slight degree of acidosis is actually produced.

But this conception neglects entirely the effects of the H and $HCO_3$ ions. Is this justifiable? We know, for example, that the H ion, within the blood at least, shifts with extreme rapidity between the cells and the plasma with every circuit of the blood and we suspect that lactic acid also leaves the tissues in the dissociated form. At least, that is to be expected if the metallic cations do not shift. If so, there appears to be no reason why dissociated carbonic acid should not also diffuse which suggests another possible stimulating effect of sodium bicarbonate.

Arrhenius (22) showed that the addition of a salt to a solution of its acid increased the rate of diffusion of the acid from the solution. For example, if we stratify a solution of hydrochloric acid with water, a definite number of chlorine ions will tend to leave the acid for the water. In so doing they will pull the hydrogen ions with them and

[3] In view of the fact that the acidity of the tissues as a whole and of the circulating blood may vary in opposite directions we have employed the terms acidosis and alkalosis to indicate an increased acidity or alkalinity of the tissues above the usual value under conditions of rest, and acidemia and alkalemia to indicate respectively increased acidity or alkalinity of the blood. It would be advantageous if data were available to similarly classify the conditions of the lymph bathing the cells particularly of the lymph in and about the respiratory center.

turn the water acid. This process is, of course, accelerated by the further addition of chlorine ions in the form of hydrochloric acid to the acid solution. But Arrhenius found that the addition of neutral sodium chloride was also effective in accelerating the diffusion of the acid. This addition increases the number of chlorine ions tending to leave the acid solution for the water, but inasmuch as the chlorine ions cannot leave by themselves, they will take either H or Na ions with them. The H ion being the more mobile is the more easily pulled from solutions and in consequence acid diffuses more rapidly than before.

With the use of membranes, Loeb (23) demonstrated the migration of hydrochloric acid from a point of lower to a point of higher concentration. As this phenomenon is independent of the membrane, it is easily demonstrated with the method of stratification which we employed above. We added hydrochloric acid to distilled water plus thymol blue as an indicator up to an acidity of pH 2. The solution was divided into two portions and sodium chloride was dissolved in one. The two solutions were than stratified with the salt and acid solution lying below. A disturbance in the hydrogen ion equilibrium occurred at the boundary as rapidly as stratification could be accomplished with a display of colors, indicating greater acidity above and lesser acidity below the original interface. With the solutions employed the acid diffused against a positive gradient of full pH I.[4]

It is obvious that the conditions are somewhat different in the case of a weak acid, such as carbonic acid, and its salt sodium bicarbonate, for the addition of the salt markedly reduces the hydrogen ion concentration of the solution. Yet in so doing it has not only increased the amount of freely diffusible undissociated $CO_2$ and $H_2CO_3$, but has increased the rate of diffusion of the H ions which have been decreased in number. So far as the acidity of the interior of the living cell placed in such a solution is concerned, the effectiveness of the remaining hydrogen ion is theoretically increased for presumably the relative impermeability of the cell membrane to the sodium ion will prevent an accelerated diffusion of the added bicarbonate. Theoretically, at least, the intravenous injection of sodium bicarbonate should exert a double effect, preventing the diffusion of carbon dioxide from the tissues into the blood, by increasing the concentration of $H_2CO_3$ and $CO_2$ in the blood and preventing the diffusion outwards from the cells of the $HCO_3$ ion to which the H ion is attached.

[4] It would appear that sodium chloride must influence the shift of hydrochloric acid between tissue fluids and cells in general. Of particular interest would be the effect on the exchange of acid between the respiratory center and the lymph, and the red blood cells and the plasma.

Although we are not unmindful of possible specific effects of various acids (24), yet it would appear from the foregoing that the possibilities of purely acid effects of carbon dioxide and sodium bicarbonate have not been exhausted. Furthermore, we find difficulty in reconciling other observations with the specific effects of carbon dioxide; for example, the effects of altitude on pulmonary ventilation. As Fitzgerald (25) has shown, there is a very definite relation between altitude and alveolar carbon dioxide—the higher the altitude the lower is the alveolar carbon dioxide tension. Yet under these conditions the lower the carbon dioxide tension in the tissues and the blood the greater is the pulmonary ventilation. Obviously, if carbon dioxide exerts a specific stimulating effect on respiration, it must be very slight.

It would therefore appear that the results of Scott (4) may be interpreted in terms of acidity and we are inclined to believe that the recent experiments of Dale and Evans (14) serve as a counterpart of the

TABLE 1

| | | NORMAL TISSUE | CARBON DIOXIDE DIFFUSION GRADIENT FROM THE TISSUE TO THE BLOOD |
|---|---|---|---|
| Normal blood | $\frac{6\ H_2CO_3}{120\ H_2CO_3}$ | $\frac{6.2\ H_2CO_3}{120\ BHCO_3}$ | 0.2 |
| Increased alkaline blood produced by excessive pulmonary ventilation | $\frac{4\ H_2CO_3}{120\ NaHCO_3}$ | $\frac{6.2\ H_2CO_3}{120\ BHCO_3}$ | 2.2 |
| Increased alkaline blood produced by the injection of $NaHCO_3$ | $\frac{6\ H_2CO_3}{150\ NaHCO_3}$ | $\frac{6.2\ H_2CO_3}{120\ BHCO_3}$ | 0.2 |

experiments of Scott. The results of Scott and of Dale and Evans may be explainable on the basis of the differential barrier offered to the shift of the numerator and denominator of the carbonate buffer system between the blood and tissues, as is shown in table 1,[5] in which the composition and acidity of the blood and tissues are roughly represented. Column 1 represents the normal blood, the blood after ventilation and after the injection of sodium bicarbonate, and column 2 the normal condition of the tissues. For normal conditions of the

[5] No attempt is made to designate the different proportion of sodium and potassium in the carbonate buffer mixtures in and outside of the cells. The fact that potassium is the predominating metallic cation in muscle cells, that lactic acid liberated in the cells reacts with the bicarbonate in the cells and that no parallel increase of potassium and lactic acid in the blood stream as a result of exercise has been described suggests at least the relative impermeability of the living cell membrane to the metallic cations and that the lactic acid enters the lymph in the dissociated form.

blood we have allotted 6 parts of $H_2CO_3$ and 120 parts of $NaHCO_3$ with a normal pH value. Not knowing the alkaline reserve of the tissues, but knowing that the concentration of carbon dioxide is higher than in the blood, we have given the values of 6.2 and 120 parts respectively for $H_2CO_3$ and $NaHCO_3$. The effect of respiration on the sodium bicarbonate of the blood is ignored. The freeing of the blood of carbon dioxide and the production of alkalemia by forced ventilation is represented by the carbon dioxide ratio. By lowering the carbon dioxide tension of the blood the diffusion pressure of carbon dioxide from the tissues to the blood is increased from 6.2 minus 6.0 to 6.2 minus 4.0. The cell membrane being at least relatively impermeable to the metallic cations, a lowering of the carbon dioxide ratio within the cell with a consequent alkalosis must necessarily occur.

On the other hand, when alkalemia is produced by the injection of sodium bicarbonate, the numerator of the carbonate buffer system remains constant. Ignoring the salt effects which we have discussed above, the diffusion pressure of carbon dioxide from the tissues to the blood has not been disturbed and consequently the rate of diffusion of carbon dioxide from the tissues is unaffected. The numerator of the carbonate buffer system in the tissues, therefore, remains the same. Since sodium bicarbonate, which is increased in the blood, presumably does not diffuse freely into the tissues, the $\frac{H_2CO_3}{NaHCO_3}$ ratio and therefore the hydrogen ion concentration of the tissues should not be materially changed. If we are prepared to accept this differential diffusion of the numerator and denominator of the carbonate buffer mixture, it is obvious that the effects of changes in the hydrogen ion concentration of the blood on the hydrogen ion concentration of the tissues will vary depending upon the method of production employed, whether it is reduced by an increase in the sodium bicarbonate or by a decrease in the carbon dioxide.

III. The hydrogen ion concentration of the arterial blood as a regulator of respiration. The hydrogen ion concentration of the arterial blood has been generally considered as the prime factor controlling respiration. Though the view has been questioned from time to time, it has in turn been vigorously defended by Winterstein (7), Hasselbalch (9), Haldane and Priestley (26), Campbell, Douglas and Haldane (27), (28) and others.

It is true that under normal conditions the relative constancy of the arterial pH and the similarity of response of various individuals to the administration of carbon dioxide (29) suggests a constancy of response of the respiratory center to the hydrogen ion of the blood. Yet, if under peculiar conditions a lack of correspondence between pulmonary

ventilation and the acidity of the blood occurs, it is of fundamental concern to know whether the threshold of stimulation has been changed, whether the excitability of the center has been altered or whether the hydrogen ion concentration of the blood bears no close or direct relation to pulmonary ventilation.

Instances are accumulating exemplifying a lack of parallelism between pulmonary ventilation and acidity of the arterial blood. Among these instances may be cited our experiments (30) on the dog showing striking changes in respiration unaccompanied by or unrelated to changes in the hydrogen ion concentration of the arterial blood. In these experiments in which we studied the effects of changes in blood volume and the total pulmonary ventilation, we found that hemorrhage increased pulmonary ventilation and that subsequent injection decreased this ventilation. The most obvious inference from such experiments is that the volume-flow of blood is an important factor controlling respiration; but what is the mechanism?

It is generally assumed that the greater the venosity of the blood coming from the tissues, the greater its acidity and, therefore, the greater the acidity of the arterial blood reaching the respiratory center. Hence the increased pulmonary ventilation following hemorrhage. If this explanation is correct, the respiratory center responds to the acidity of the arterial blood which in turn is determined by the rate of metabolism of the tissues as a whole and by the volume-flow of blood through the tissues.

But we noted that the changes in ventilation produced by the injection of the gum-saline solution occur promptly with changes in blood pressure; for example, hyperpnea may be immediately transformed to apnea by injection. That is, unmodified blood already through the tissues when accelerated through the respiratory center changes respiration. Obviously, the apnea cannot be attributed to a reduction in the hydrogen ion concentration of the arterial blood, nor can it be attributed to a reduction of the total number of free hydrogen ions brought per unit of time to the center, as the latter are actually increased by injection. If the regulation of respiration is determined by the hydrogen ion it would, therefore, seem more logical to consider the effects of the arterial blood in relation to the transport of carbon dioxide and hydrogen ions away from the site of action rather than the transport of free hydrogen ions to the center. And in this connection it should be mentioned that the transport of acid from the tissues is not directly dependent on the hydrogen ion concentration of the blood.

The conception of the significance of the transport of acid from, rather than to, the respiratory center involves the acid metabolism of the respiratory center itself. There is much information that is in

accord with this view, as will be pointed out below. But for the present we conclude from our experiments that not only is the metabolism of the body as a whole a factor in the chemical control of respiration, but also and to an important degree the metabolism of the respiratory center itself. The ultimate effect of the volume-flow of blood, that is, the acidity of the center determined by the composition of the blood, the volume-flow of blood through the center and the metabolism of the center itself, is the factor controlling respiration.

The apnea elicited by the injection of gum-saline solution may last a minute or more, but when respiration is reëstablished less air is moved than during the period of depleted blood volume. This phenomenon is of considerable interest, for the intravenous injection of gum-saline solution must of necessity dilute the buffers and carbon dioxide carriers of the blood.

It is true that simple dilution of the relatively acid red blood cells may decrease the acidity of the blood enough to slightly depress respiration. Parsons (31) states that an increase in the red cells from 5,000,000 to 7,000,000 per c. mm. should increase the acidity of the blood sufficiently to increase respiration 43 per cent. Parson's statement is, of course, based on the generally accepted view that the hydrogen ion concentration of the blood stimulates the respiratory center.[6] In this connection it should also be mentioned that gum-saline solution is acid relative to blood. Whether this acidity counteracts the alkaline effect of dilution we do not know, yet we are convinced that the hydrogen ion concentration of the blood bears no important relation to the decreased respiration following injections for the reason that the most pronounced effect, apnea, was elicited by unchanged blood and that the subsequent period of decreased respiration lasts only ten to fifteen minutes though dilution of the blood is maintained much longer. The depression of respiration is attributable to the generally beneficial effect of the injection of a non-nutrient solution. It increases the volume-flow of blood out of proportion to the dilution of the blood, thereby increasing the nutrient flow. In this particular instance the increased flow of carbon dioxide carriers is significant, and is in line with our previous suggestion that neither the number of H ions brought to the center nor the concentration of the hydrogen ions of the arterial blood determines the extent of ventilation, but rather the efficiency of the blood and lymph as carriers of the hydrogen ions away from the site of action.

These views seem to be supported by other experiments. Take, for example, the respiratory response to room air following a period of

[6] It is interesting to recall that the increase in the red blood cells during acclimatization to high altitudes is associated with decreased pulmonary ventilation.

anoxemia. It is known that oxidation of the hemoglobin of the blood increases the acidity of the blood (32) even though the carbon dioxide tension remains constant. How then are we to interpret the well-known apnea or depressed respiration on breathing room air after hyperpnea elicited by a decreased oxygen tension of the respired air? Since the pulmonary ventilation is not altered until the more highly oxygenated blood has reached the respiratory center, it is safe to assume that the alveolar carbon dioxide tension obtaining in the lungs is not lowered at the time of the increased oxidation of the hemoglobin. Moreover, we know that the oxidation of hemoglobin drives carbon dioxide from the blood and thereby increases the alveolar carbon dioxide tension (33). Despite the increased acidity of the blood from oxidation of the hemoglobin and from exposure to increased alveolar carbon dioxide, apnea occurs at the moment the acid blood reaches the center.

The similar effects on pulmonary ventilation of the administration of carbon monoxide and of nitrogen also are of significance. Though the administration of carbon monoxide, like oxygen, increases the acidity of the arterial blood and the administration of nitrogen decreases the acidity, both gases elicit comparable hyperpnea.

Other examples of lack of parallelism between pulmonary ventilation and the hydrogen ion concentration of the arterial blood are the hyperpnea of lack of oxygen in the respired air (2), (3), of anemia (34) and of cardiac decompensation (35) all of which may be associated with increased alkalinity of the arterial blood.

Similar discrepancies may occur as a result of the intravenous injection of alkali and acid as more recently shown by Mellanby (36). He found that while the administration of carbon dioxide and nitrogen markedly increase respiration with little change in the reaction of the blood, the injection of alkali or acid produces large changes in the hydrogen ion concentration with but little effect on respiration. These results from carbon dioxide agree with those of Haldane and Priestley (26) who, as a result of administration of carbon dioxide, describe an astounding sensitivity of the respiratory center to changes in the hydrogen ion concentration of the blood. The results from the lack of oxygen are in agreement with the tendency of anoxemia to produce alkalemia as found by Haggard and Henderson (2) and Haldane, Kellas and Kennaway (3). The effects of injection of alkali and acid agree with those of Laquer and Verzar (8), Hooker (10), Scott (4), Dale and Evans (14) and others. It appears, therefore that certain fundamental points are fairly well established supporting our view on the parallelism of respiration and the acidity of the respiratory center as opposed to the acidity of the arterial blood.

The question now arises, what is the explanation of the lack of correspondence between the hydrogen ion concentration of the blood and respiration? Since the hydrogen ion concentration of the tissues and the tissue fluids is determined by the amounts of weak acids and weak bases present, being a function of the law of mass action, it obviously follows that diffusion gradients of acidity and alkalinity as such do not exist between the blood and tissues, for acidity and alkalinity are conditions dependent on more than one substance each having a different rate of diffusion. The resultant effect of the amount of diffusion of the various acids and bases will therefore determine the hydrogen ion concentration at any point in the tissues between the interior of the cell and the blood.

Since the administration of carbon dioxide increases its tension in the blood, it obviously decreases the carbon dioxide diffusion gradient from the tissues to the blood. The immediate effect is increased acidity of the tissues including the respiratory center. And similarly, if one recalls that lack of oxygen leads to greater formation of acid at the site of action and that excitations of the center tend to lower the hydrogen ion concentration of the blood by excessive ventilation, the apparent sensitivity of the respiratory center to the hydrogen ion concentration of the blood is again explained. Under these two conditions it is only necessary to assume a high sensitivity of the respiratory center to the hydrogen ion to explain large fluctuations in pulmonary ventilation associated with small changes in the acidity of the blood.

On the other hand, when the acidity of the blood is changed by varying the amount of sodium bicarbonate in it, either by the injection of sodium bicarbonate or hydrochloric acid, the conditions are different, as was pointed out in connection with the discussion of the specificity of carbon dioxide. Under these conditions, ignoring the temporary liberation of carbon dioxide on the administration of acid, the diffusion gradient of carbon dioxide from the tissues to the blood is altered very little. Consequently, if the tissues are impermeable or relatively impermeable to sodium bicarbonate the $\frac{H_2CO_3}{NaHCO_3}$ ratio of the tissues remains unchanged despite the change occurring in the blood. Theoretically at least, it seems not improbable that relatively large changes in the acidity of the blood may be produced without the accompaniment of similar changes of acidity in the tissues.

The relation of the alkaline reserve of the blood to respiration is, of course, not as simple as assumed above, for we know that respiration increases with progressive injection of hydrochloric acid. Whether this increased respiratory response is due to decreased buffer properties of the lymph about the nerve elements of the respiratory center which

would increase the effectiveness of the liberated carbon dioxide in raising the hydrogen ion concentration at the cell membrane, or whether to a gradual decrease in buffers of the center itself, still remains to be determined. Several years ago (37) we found that equal injections of hydrochloric acid separated by equal intervals of time elicited progressively smaller and smaller diminution in the alkaline reserve of the blood until the acid injected was completely neutralized. Whether any of the base which neutralized the acid was derived from the respiratory center we do not know.

IV. The oxygen tension of the arterial blood and the coördination of the dual function of hemoglobin as regulators of respiration. During asphyxia two important chemical changes occur within the body, a progressive increase in the carbon dioxide tension of the blood and tissues and a progressive decrease in the oxygen tension. Whether the increased stimulation of respiration is due to the increased acidity from the accumulated carbon dioxide and lactic acid, or whether it is due to an effect of lack of oxygen unrelated to acidity, has been vigorously debated and is still regarded an open question.

The problem is exceedingly involved. The composition of the blood is undoubtedly an important factor regulating pulmonary ventilation, but a critical analysis of the relation of any single property of the blood, which presumably influences respiratory activity such as the hydrogen ion concentration, the carbon dioxide tension and the oxygen tension of the arterial blood, has in each case revealed a gross lack of correspondence. Yet a consideration of such factors as the lack of correspondence between tissue acidity and the acidity of the blood, the volume-flow of blood, the metabolism of the respiratory center itself and the significance of the coördination of the dual function of hemoglobin which have been largely overlooked perhaps will aid us in an ultimate interpretation of existing data.

Though it has been suggested by Douglas and Haldane (38), (39), (40) and Mathison (41), (42) that lactic acid may accumulate within the respiratory center itself as a result of anoxemia, this view has received little attention compared with the effects of acid metabolites delivered by the tissues as a whole to the respiratory center via the blood stream (43), (44) (45). Geppart and Zuntz (45) first emphasized the importance of metabolites in the circulating blood arising from reduced oxidation, and abundant evidence of the formation of lactic acid under these conditions has now accumulated. Boycott and Haldane (43) state that the hyperpnea from lack of oxygen is probably due to a gradual formation of lactic acid or other substances which have the same influence as carbon dioxide on the respiratory

center so that the threshold value of stimulation of carbon dioxide is lowered.

More recently these views on the indirect acid effects of lack of oxygen have been questioned. The Anglo-American Expedition of Pike's Peak (46) found no evidence of abundant formation of lactic acid which might account for low alkaline reserve accompanying anoxemia. Later Haggard and Henderson (2) and Haldane, Kellas and Kennaway (3) independently reached the conclusion that the low alkaline reserve of the blood during anoxemia is not due to acidosis, but is virtually an alkalosis produced by the washing out of carbon dioxide, and in his recent book on respiration Haldane (1) appears to have abandoned his earlier views on the significance of lactic acid as a respiratory hormone. Haggard and Henderson deny the occurrence of acidosis in anoxemia and state that the formation of lactic acid within the body should depress rather than increase respiration.

In the Anglo-American Expedition to Pike's Peak it was further noted that relatively slight and localized exertion produced marked hyperpnea and the theory was proposed by Haggard and Henderson and by Haldane (5) that the hyperpnea of anoxemia may be due to imperfectly oxidized metabolites which, on account of the low oxygen pressure of the blood, pass the lungs in greater amount and stimulate the respiratory center. This factor in the control of respiration, comparable to that suggested by Pflüger (47), has been designated by Haggard and Henderson as Respiratory X.

We have mentioned the older view of Haldane and his associates that lack of oxygen lowers the threshold of stimulation of the respiratory center to carbon dioxide. Linhard (48), on the other hand, finding an increased respiratory response to the administration of carbon dioxide with decreasing oxygen tension, concludes that the *excitability* of the center toward the stimulus is increased by lack of oxygen in the respired air. A similar conclusion is reached by Hasselbalch and Lundsgaard (49) who find a decreased pulmonary ventilation on the administration of oxygen despite the fact that oxygen lowers the pH of the blood. They state that their experiments speak unequivocally for the fact that the "Erregbarkeit" of the respiratory center for its adequate stimulus, the carbon dioxide or the hydrogen ion concentration of the blood is variable and in inverse proportion to the oxygen tension. Milroy (50) also concluded from direct observations on the hydrogen ion concentration of the arterial blood in periodic respiration that the excitability of the center as gauged by its threshold of excitability to the hydrogen ion is evidently lowered by increased absorption of oxygen.

In contrast to the preceding theories is the theory of Rosenthal (51), in accordance with which lack of oxygen acts as direct stimulus to the respiratory center as exemplified in apnea following forced pulmonary ventilation. Although Rosenthal failed to explain the apnea following forced ventilation with nitrogen and hydrogen (52) and the absence of apnea following normal respiration of pure oxygen, the recent experiment of Gregg, Lutz and Snyder (53,) (54), (55) and others demonstrating the extreme sensitivity of the center to changes in the oxygen tension of the respired air has revived the interest in Rosenthal's theory. The objection raised by many workers (56), (10), (57), (50), (58), (59) to the indirect acid effect of lack of oxygen is that the time between the onset of lack of oxygen and increased respiration is insufficient for the adequate accumulation of acid.

Though there is as yet no agreement as to the mechanism of the chemical control of respiration, yet its relation to the supply of oxygen and the transport of acid metabolites seems established. Both of these functions are intimately associated with tissue metabolism and consequently the solution of the chemical control of respiration seems to require a consideration of the acid metabolism of the respiratory center, of the volume-flow of blood and of the coördination of the dual function of hemoglobin.

For some years past we (60), (61), (62), (63), (64), (65), (66), (67), (68) have been interested in the relation of the volume-flow of blood to tissue metabolism and there is every reason to believe that our findings are directly applicable to the metabolism of the respiratory center. We found that hemorrhage decreased and that subsequent injection of gum-saline solution increased the volume-flow of blood (61) out of proportion to the changes of blood volume entailed (62), (63), from which it necessarily follows that hemorrhage decreases and injection increases the rate of flow of the various blood constituents through the tissues. We found that a reduction of the volume-flow of blood elicited by hemorrhage decreased the response of the submaxillary gland to pilocarpin and that subsequent injection of a non-nutrient solution elicited a prompt and striking acceleration of secretion (63), (66). Similarly the reduced response of the sartorius muscle to tetanic stimulation elicited by hemorrhage was almost completely removed by subsequent injection of gum-saline solution (66).

The harmful and beneficial effects of the retardation and acceleration of the flow of blood are thus established, but whether these effects are due to a general disturbance of the supply of all nutrient materials and to a similar disturbance of the transport of all metabolites from the tissues, we are not prepared to say. The fact that the amount of oxygen in solution in the tissues is extremely small, and that the amount of

acids storable without gross change in the hydrogen ion concentration is likewise small, indicates that much of the disturbance may be attributable to changes in the transport of carbon dioxide and oxygen.

We therefore studied the relation of blood volume to the transport of carbon dioxide and oxygen (67), (68), and in the interpretation of our results found that we had unwittingly become involved in the long-debated and far more difficult problem of the chemical control of respiration.

We followed the transport of carbon dioxide by recording the pulmonary ventilation during the administration of room air and of a mixture of carbon dioxide in room air before hemorrhage, after hemorrhage and after the injection of gum-saline solution. We assumed, merely as a working basis, that the formation of carbon dioxide in the tissues progresses at a constant rate and that ample time exists for the unloading of the major portion of carbon dioxide as the blood passes through the lungs. Therefore, the more frequent the passage of the blood from the point of loading to the point of unloading the lighter the load of carbon dioxide as the blood leaves the tissues and presumably the less is the respiratory center stimulated. Likewise, the lighter the total load of carbon dioxide of the blood as it passes through the respiratory center, the less will be the total respiratory stimulus when the elimination of carbon dioxide is hampered by its administration. Assuming that the respiratory center serves as a reliable index to the carbon dioxide or the hydrogen ion concentration of the blood we have a ready means of testing the effects of hemorrhage and of infusion of gum-saline solution on the volume and nutrient flow of blood. Experiments showed that hemorrhage increased the pulmonary ventilation on the administration of room air and of a mixture of carbon dioxide in room air and that subsequent injection of gum-saline solution reduced this ventilation.

As has been indicated, the injection of gum-saline solution has two effects. It increases the volume-flow of blood and it dilutes the blood. This dilution necessarily entails a dilution of the carbon dioxide carriers and buffers. It therefore seemed that if the injection of gum-saline solution, which decreases the carbon dioxide carrying properties of the blood, still decreases the respiratory response to the administration of carbon dioxide, it must improve the transport of carbon dioxide and lower the carbon dioxide tension in the tissues through the improvement of the nutrient flow primarily of carbon dioxide carriers.

Such conclusions are obviously open to objection for they neglect the effects of blood volume on the transport of oxygen. According to Van Slyke (69), hemoglobin is almost as responsible for the transport of carbon dioxide as of oxygen. And our experiments show that

exceedingly small hemorrhages elicit a definite reduction in the rate of oxygen consumption; that subsequent injection of gum-saline solution increases the rate of oxidation.

The experiments, therefore, are no more crucial than a host of others in determining whether lack of oxygen acts directly or indirectly. The fact that the administration of carbon dioxide elicits greater ventilation after hemorrhage than before might be explainable as an effect of lack of oxygen on the excitability (48), (49) of the center to its normal stimulus, carbon dioxide. On the other hand, the diminution of oxidation considered in relation to the acid metabolism of the respiratory center, the broken coördination of the dual function of hemoglobin with its impaired transport of acid from the respiratory center suggests a more effective mechanism of acid control than has hitherto been described.

Christiansen, Douglas and Haldane (32) showed that when oxyhemoglobin loses oxygen it acquires more basic properties. They called attention to the significance of this phenomenon to the transport of carbon dioxide and to the hydrogen ion concentration of the venous blood. Under normal conditions the carbon dioxide liberated by the tissues approximately neutralizes the alkali liberated by the reduction of the oxyhemoglobin, and the hydrogen ion concentration of the venous and arterial blood are very nearly equal. From the results of Christiansen, Douglas and Haldane it must necessarily follow that if the tissues fail to absorb oxygen and thereby fail to reduce oxyhemoglobin they fail to provide themselves with a vehicle for the export of acid which continues to be formed. It is readily understood why a rapid accumulation of acid in the tissues might occur under these conditions. This failure of hemoglobin to provide the tissues with an adequate amount of oxygen and thereby provide them with the normal vehicle for the transport of acids we have designated as broken coördination of the dual function of hemoglobin. The effects of this broken coordination on the carbon dioxide tension and hydrogen ion concentration are shown below.

According to Christiansen, Douglas and Haldane, the average oxygen capacity of 100 cc. of blood is 18.5 cc. Blood which is 96 per cent saturated would, therefore, hold 17.76 cc. of oxygen. If all of this oxygen is consumed about 15 cc. of carbon dioxide would be formed and if this were taken up by the blood in the absence of reduction of oxyhemoglobin, the carbon dioxide pressure would rise from 40 to 87 mm.; in the presence of reduction from 40 to 62 mm., or a total of only 22 mm. Assuming that about one-third of the oxygen is lost by the blood the carbon dioxide pressure would rise only 7 mm. in the blood instead of $\frac{47}{3}$ or 18 mm.

Recently L. J. Henderson (70) has reached the following conclusion in relation to the hydrogen ion concentration of the blood.

> Again passing from oxygenated blood with a tension of $CO_2$ of 40 mm. to reduced blood with a tension of $CO_2$ of 60 mm. involves a change of hydrogen ion concentration from $4.62 \times 10^{-8}$n to $5.65 \times 10^{-8}$n: the increase of combined carbonic acid is 62.0 − 48.8 = 13.2 per cent by volume; the base yielded by the hemoglobin radical is 14.6 − 5.2 = 9.4 per cent by volume and that yielded by ordinary buffer action only 13.2 − 9.4 = 3.8 per cent by volume. In this last case, if the peculiar function of hemoglobin were eliminated, the change in absorption of carbonic acid for the given change of hydrogen ion concentration would be only $\frac{3.8}{13.2}$ = 29 per cent of its real value. The change in the hydrogen ion concentration for the given change in carbonic acid, however, would be at least 350 per cent of the real value and the concentration of the hydrogen ion would rise to more than $8 \times 10^{-8}$n.

Whether the respiration is a response to the hydrogen ion concentration of the blood, or of the center itself, or of the carbon dioxide tension of the arterial blood, the coördination of the dual function of hemoglobin must be a very significant factor. Haldane showed that a rise of arterial carbon dioxide tension of 1.5 mm. Hg is sufficient to double pulmonary ventilation. On the basis of these figures and those of Christiansen, Douglas and Haldane, the absence of the normal reduction of hemoglobin which would increase the carbon dioxide tension 11 additional mm. Hg would elicit a 700 per cent increase in respiration.

So far as we are aware, the important findings of Christiansen, Douglas and Haldane have not been applied to hyperpnea due to lack of oxidation in the respiratory center. Yet it is very likely that the respiratory center suffers from the lack of oxygen just as do other tissues. It is only by its increased activity from increased acidity that a general reduction in oxygen consumption is prevented. At least in the experiments on hemorrhage, in which adequate compensations against lack of oxygen cannot occur, the decided decrease in the total oxygen consumption from relatively small hemorrhages is suggestive of a general reduction in oxygen consumption including that of the respiratory center as well as the tissues as a whole.

Nevertheless it might be objected that the hyperpnea of hemorrhage is not due to a disturbance in the dual function of hemoglobin, but rather to a reduction in the flow of carbon dioxide carriers and buffers. This objection, however, is met by our experiments on blood volume and tissue nutrition (68).

In these experiments we studied the effects of saturation of hemoglobin with carbon monoxide on the response to carbon dioxide and on oxygen consumption. The administration of monoxide undoubtedly accelerates the volume-flow of blood. Yet carbon monoxide increased

pulmonary ventilation and decreased the rate of oxidations. The experiments are, therefore, suggestive of a disturbed transport of carbon dioxide despite a flow of carbon dioxide carriers above normal. Our view on the significance of the liberation of carbon dioxide carriers consequently holds for conditions of normal blood volume as well as deficient blood volume.

As confirmatory evidence of the significance of the dual function of hemoglobin we sought to find other means of disturbing its coördination. It is suggested that the stimulating effect of sodium cyanide is another example of impaired transport from the tissues. Though an abundant supply of oxygen to the tissues prevails, the inability to utilize it prevents the usual liberation of the potential alkalinity of the blood. But unfortunately this as well as every other method of disturbing the dual function of hemoglobin involves a reduction in oxidations.

An exception to this may be hyperpnea of oxygen-poisoning in which neither the supply of oxygen nor the ability of the tissues to utilize oxygen may be primarily involved. When blood is subjected to three atmospheres of pure oxygen, it takes up in addition to the 20 volumes per cent combined with hemoglobin, approximately 6 cc. in a simple solution. This additional 6 cc. is the normal amount of oxygen lost in the tissues per 100 cc. of circulating blood. Since oxyhemoglobin cannot be reduced until the excess of oxygen in solution is absorbed by the tissues, it follows, other things remaining constant, that an animal subjected to three atmospheres of pure oxygen is totally robbed of the potential alkalinity of its hemoglobin. Since three-fourths of the alkali liberated in the blood to carry and buffer the carbon dioxide is derived from the reduction of oxyhemoglobin (69), the transport of carbon dioxide should be markedly disturbed.

Supporting this view are the asphxial symptoms of animals subjected to high pressures of oxygen. Bert (71) states that the severity and suddenness of onset of symptoms vary with the tension of oxygen. They are severe at two atmospheres of oxygen, the animals showing the usual symptoms of air hunger. When subjected to about four atmospheres of pure oxygen they are abrupt and asphyxial, the animals showing strychnine-like convulsions. Smith (72), Hill (73), (74), Hill and Macleod (75), (76) and the author obtained comparable results.

At the same time Bert, Panum (77) and Hill and Macleod find decreased elimination of carbon dioxide, due as they believe to the arrest of oxidative processes in the tissues by the high partial pressure of oxygen.

This view is generally accepted and is employed by Dautrebande and Haldane (78) as a working basis to determine circulatory com-

pensations protecting the highly sensitive central nervous system from high oxygen pressures. They find that the reduction of 3.5 mm. in the alveolar carbon dioxide of man subjected to two atmospheres of pure oxygen corresponds to a slowing of the brain circulation sufficient to reduce to normal the oxygen pressure in the blood leaving the brain.

In view of the possible disturbance of the transport of acid the problem of oxygen poisoning needs re-analysis. Yet there are miscellaneous data suggesting the nature of oxygen poisoning. For example, it may be only accidental, but nevertheless interesting, that the marked and sudden symptoms of air hunger occurring in animals possessing hemoglobin occur at approximately the theoretical oxygen pressure which completely eliminates the liberation of the potential alkalinity of the hemoglobin. Furthermore, it may be of significance that animals whose transport of carbon dioxide is dependent on hemoglobin when exposed to one atmosphere of oxygen are harmfully affected, whereas tissue cultures independent of circulating hemoglobin for the elimination of carbon dioxide are immune (79). A muscle nerve preparation subjected for several hours to 50 or 60 atmospheres of oxygen may show little signs of injury (73). The extreme sensitivity of the central nervous system to increased acidity agrees with its sensitivity to lack of oxygen as well as excess of oxygen. Whereas, the response of the heart (80) and pulmonary ventilation to the administration of high oxygen tension is prompt, Hill and Macleod found the reduced oxygen consumption delayed. Such results might imply that reduced oxidations are secondary to the development of acidosis.

All of these observations seem to indicate that oxygen poisoning is explainable by the development of acidosis. On the other hand, Bert found when animals were placed in a confined space and subjected to one to five atmospheres of air, that the tension of carbon dioxide when death occurred was about 25 per cent; but when the animals were subjected to higher pressures the lethal concentration of carbon dioxide was considerably less. As similar results were obtained with approximately one-fifth the pressures when pure oxygen was employed, he concluded that neither the carbon dioxide nor the barometric pressure but rather the high tension of oxygen was the determining lethal factor.

Yamada (81) noted in man that a continuous administration of a 3 per cent mixture of carbon dioxide in room air elicited a distinct hyperpnea which tended to diminish. When the same percentage of carbon dioxide was administered in pure oxygen the hyperpnea tended to increase. This led Yamada to conclude that pure oxygen at one atmosphere exerts a beneficial effect on the respiratory center, increasing its excitability.

We have arrived at opposite conclusions from experiments on the rat. These experiments were based on the reasoning that if the primary disturbance of oxygen poisoning is the impaired transport of acid, animals subjected to high oxygen tension should be very sensitive to the administration of carbon dioxide. The toxicity of various gas mixtures at similar combined pressures were tested in a compression chamber which was composed of an iron casing about 18 cm. in diameter and 55 cm. long fitted at each end with a movable glass plate window and provided with a series of stop cocks for the delivery and sampling of gases, for the registration of pressures, etc.

In one of the experiments three white rats, of nine litter mates about two months old, were placed in the chamber in an atmosphere of 40 per cent of oxygen and 60 per cent nitrogen. The barometric pressures was then raised with nitrogen to 2700 mm. Hg. By a special arrangement a solution of sodium carbonate was dropped in a bottle of sulphuric acid at one end of the chamber. The carbon dioxide which was evolved was distributed by means of a perforated tube running the full length of the ceiling of the casing. Sufficient gas was evolved to give a 5 per cent mixture by volume of carbon dioxide at 760 mm. Hg barometric pressure. The purpose of this experiment was to test the effects of increased carbon dioxide plus increased barometric pressure. The initial 300 mm. Hg of oxygen were administered to avoid excessive reduction of the pressure of oxygen from a prolonged experiment. The hyperpnea elicited by the carbon dioxide was well maintained and, aside from a certain degree of restlessness, the animals remained in good condition. At the end of four hours the experiment was discontinued.

Three other rats were then subjected to 2700 mm. Hg of pure oxygen. This elicited a definite hyperpnea which increased as the experiment progressed, but aside from this and a little restlessness the animals appeared fairly normal at the outset. They washed themselves assiduously and from time to time would take short naps, but gradually the restlessness increased. At the end of three and one-half hours the rats were decompressed, for one began to show signs of distress. All recovered promptly.

The last three rats were then subjected to the same pressure of pure oxygen for one-half hour during which time they behaved just as did the second trio. Carbon dioxide equal in amount to that administered to the first trio was then given. Very marked dyspnea, exceeding that produced by carbon dioxide alone or higher tension of oxygen alone, occurred immediately. The animals became extremely restless and in fifteen minutes were in a far more serious condition than the rats in either of the two preceding experiments. At the end of one-half hour

they were at the point of dissolution and were decompressed. Only one recovered.

From these experiments we conclude that oxygen at high tension increases the sensitivity of the animal to the administration of carbon dioxide and infer that the increased sensitivity to carbon dioxide is due to a broken coördination of the dual function of hemoglobin producing a disturbed transport and elimination of carbon dioxide. This leads to tissue acidosis which is further augmented by the administration of carbon dioxide.

The practical application of these findings to caisson sickness is obvious. The significance of the coördination of the dual function of hemoglobin in this condition has been overlooked. Hill (74) is inclined to believe that 1 to 2 per cent of carbon dioxide is of no significance; on the other hand, Snell (82) states that carbon dioxide even in relatively small amounts accentuates the injurious effects of high oxygen tension and describes a striking diminution of sickness amongst caisson workers through increased ventilation.

Our experiments also suggest a basis for determining the optimum tension of oxygen for therapeutic administration.

It may be concluded on the basis of the foregoing discussion that the significance of oxygen as a regulator of pulmonary ventilation is increased by its effects on the dual function of hemoglobin. Any conditions which prevent the reduction of hemoglobin in the tissues or its oxidation in the lungs, seriously interfere with the transport and elimination of acid with a resultant acidosis and respiratory stimulation.

V. LACTIC ACID AND THE ACID METABOLISM OF THE RESPIRATORY CENTER AS REGULATORS OF RESPIRATION. It has been stated that variation in the volume-flow of blood through the respiratory center affects pulmonary ventilation; that hyperpnea elicited by hemorrhage may be transformed to apnea by the acceleration of unchanged blood through the center. These findings point to the metabolism of the respiratory center as one of the factors regulating respiration, for the so-called *stimulating* factors of carbon dioxide, of the hydrogen ion and of the oxygen of the arterial blood remain unchanged.

Apnea thus produced may be due to several causes. By accelerating the flow of the carbon dioxide carriers, the carbon dioxide diffusion gradient from the center to the blood is increased. By improving the transport of oxygen, oxidations are increased and lactic acid is more completely removed. By increasing the rate of oxygen consumption more potential alkalinity of the hemoglobin is liberated and the carbon dioxide diffusion gradient is increased still more.

Each of these causes involves the metabolism of the respiratory center. But on the whole this factor has been overlooked as a result

of the emphasis placed on the *stimulating* effect of the various blood constituents. As was indicated above, the respiratory center by virtue of the apparent delicacy of its response to stimulating agents circulating in the blood, has been viewed as a mechanism for sampling the blood rather than as a physiological unit dependent like other tissues for its sustenance on the blood, varying its own activity by variations in its own acid metabolism controlled by its own nutrient flow.

From time to time various workers have suggested that lactic acid (and fluctuations of the amount of lactic acid in the center are an index to altered metabolism) developed within the respiratory center is a regulatory factor of respiration. Yet on the whole the view has not been generally accepted and the recent demonstration of alkalemia and the absence of any appreciable amount of lactic acid in the blood during anoxemia has greatly minimized the importance of lactic acid. Haldane (1) states in his recent book on *Respiration* that the excitation of the respiratory center "by chemical stimuli depends more upon the character of the blood supplied to it than on substances generated by its own local metabolism. Thus the temporary diminution of blood supply in fainting does not produce the same prompt effect on the center as changes in the arterial blood owing to imperfect aeration in the lungs. In this respect the center is very well suited to fulfill the function of taking a part in controlling the *quality* of the general arterial blood supply of the body. The *amount* of the arterial blood is controlled in other ways."

To be sure, respiration may be altered in the absence of a causal change in the acid metabolism of the respiratory center. The carbon dioxide factor of the blood is always present and any variation will tend to exert its effect on respiration, but superimposed upon this variable factor, which is a result of the general metabolism, is the metabolism of the respiratory center itself. Perhaps a clue can be gained with respect to a possible influence of local metabolism upon respiration through a consideration of the acid metabolism of striated muscle.

The production of lactic acid in resting muscle under anaerobic conditions and its removal in the presence of oxygen (83), (84), (85), (86), its rapid formation during contraction and removal during rest (87) are well-known facts which along with the recent quantitative results of Meyerhof (88), (89) appear to be of utmost significance in explaining the regulation of respiration. Meyerhof finds, in general agreement with Hill, that only a small part of the lactic acid which accumulates in the resting muscle under anaerobic conditions is oxidized in the presence of oxygen, the remainder being transformed into its precursor. Thus for every four, five or six molecules of lactic acid, depending upon the condition of the muscle, only one molecule of acid

is oxidized forming in the process three molecules of carbonic acid. The remaining lactic acid[7] is transformed into its precursor.

From these figures we draw two conclusions relative to the total acidity of the muscle cell during anoxemia and subsequent replenishment of oxygen. For every four, five or six molecules of lactic acid which fail to disappear as a result of anoxemia one, two or three extra molecules of acid will accumulate over and above the normal amount in the presence of oxygen. And conversely, for every four, five or six molecules of excess lactic acid which disappear on the replenishment of oxygen there will be a reduction in the total acidity represented by the disappearance of either one, two or three molecules of acid. These effects of anoxemia and replenishment of oxygen are illustrated by schematic ratios. The normal reaction of the tissues is represented by the ratio $\frac{1H_2CO_3}{20\ NaHCO_3}$. It is assumed that for every five molecules of lactic acid removed only one is oxidized. If two molecules of lactic acid fail to be removed, they will combine with sodium bicarbonate and reduce the denominator to 18, for sodium lactate compared with sodium bicarbonate has very weak buffer properties.

$$\frac{1\ H_2CO_3}{20\ NaHCO_3} \qquad \frac{1\ H_2CO_3}{18\ NaHCO_3 + 2\ NaL} \qquad \frac{3\ H_2CO_3}{18\ NaHCO_3 + 12\ NaL}$$

This change is represented in the second ratio. But the reduction of the denominator entails the liberation of 2 molecules of $H_2CO_3$ which are added to the numerator. The original ratio of 1/20 is, therefore, changed to 3/18. Now beginning with anoxic conditions with an excess of sodium lactate but with the same hydrogen ion concentration, an increase in the oxygen supply leads to the reverse condition.

$$\frac{3\ H_2CO_3}{60\ NaHCO_3 + 5\ NaL} \qquad \frac{1\ H_2CO_3}{65\ NaHCO_3}$$

In the removal of 5 molecules of the lactate anion 1 is oxidized to 3 molecules of $H_2CO_3$ and the remaining 4 are returned to their precursor state. This change liberates 5 Na ions 3 of which combine with the 3

[7] Though virtually most of the lactic acid is present in the form of sodium or potassium lactate, it is usually designated as lactic acid. Even though it occurs primarily in the form of a salt it still exerts an acid effect. On reacting with $BHCO_3$ the acid forms a very weak buffer salt which replaces the more effective alkaline $BHCO_3$. It therefore reduces the $BHCO_3$ and at the same time increases the acid numerator ($H_2CO_3$) of the carbonate buffer system. In this manner the amount of lactate formed or removed regulates the $\frac{H_2CO_3}{BHCO_3}$ ratio and thereby the pH.

freshly formed $H_2CO_3$ molecules leaving 2 ions unsatisfied. These combine with 2 $H_2CO_3$ molecules of the numerator of the buffer system. The original ratio of 3/60 changes to 1/65.

Lack of oxygen, therefore, has a double effect on the carbonate buffer system of the muscle cell. It increases the numerator and decreases the denominator with a consequent increase in acidity, while a subsequent abundance of oxygen increases the denominator and decreases the numerator with a consequent increased alkalinity.

The questions which arise in connection with these facts are, whether lactic acid can be looked upon as a normal metabolite, whether it is always present in the tissues but in varying amounts depending upon the supply of oxygen, and whether a similar formation and removal of lactic acid occurs in the acid metabolism of the respiratory center.

There can hardly be any doubt as to the answer of the first two questions. Verzar (90) has found that a slight reduction in the volume-flow of blood through muscle decreases its rate of oxygen consumption, indicating that normally the oxygen pressure in the muscle cells is very low. Krogh (91) has noted that most of the capillaries in the resting muscle of the frog and guinea pig are closed to the passage of blood. He states that a lack of oxygen undoubtedly is a frequent and probably a normal occurrence. The accumulation of lactic acid in the blood under a variety of conditions has been frequently described (92), (93), (94), (95), (96), (97), (98), (99), but its appearance in the blood of normal individuals is of special interest (100), (101).

In connection with the more direct question, Langendorff (102) found that the brain turns acid very promptly when its blood supply is cut off and Hopkins (39) states that lactic acid is found in the central nervous system as a consequence of want of oxygen and disappears in the presence of free oxygen just as in the case of muscle. The significance of lactic acid in general metabolism has been emphasized by Lusk (103), (104) and others. Dakin (105) states that "The mutual interconversion of lactic acid, glucose and glycogen seems to play a part in the metabolism of most tissues;" that "The main path of normal glucose metabolism undoubtedly lies by way of lactic acid and the conversion of lactic acid does not involve oxidation but intramolecular rearrangements."

It seems justifiable, therefore, to accept the more exact data on striated muscle as representative of acid metabolism in the respiratory center. Furthermore, this type of metabolism appears to be consonant with the effects of oxygen supply on pulmonary ventilation.

In testing this question attention is first directed to our experiments in which hemorrhage elicited hyperpnea and subsequent injection of gum-saline solution a reduction in pulmonary ventilation. In accord-

ance with the view expressed above the decreased volume-flow of blood leads to an increased rate of acid formation accompanied by a hampered transport of acid. The result is an increased acidity of the respiratory center with increased ventilation. Aside from the decreased flow of blood the conditions of hyperpnea from lack of oxygen in the respired air are probably similar. The fact, however, that such hyperpnea is accompanied by increased alkalinity of the blood has recently discounted the effect of the hydrogen ion. But this lack of correspondence between pulmonary ventilation and the pH of the arterial blood seems explainable. If the acidity of the center rather than of the blood is the factor determining the extent of pulmonary ventilation, the increased pulmonary ventilation could easily produce alkalemia by excessive elimination of carbon dioxide.

It was pointed out before that the changes of acidity of the blood as increased by the intravenous injection of acid need not necessarily be accompanied by equal changes in the tissues and thus the lack of correspondence between the hydrogen ion concentration of the arterial blood and pulmonary ventilation was explained. Likewise, other things remaining constant, the acidity of the tissues as increased by increased accumulation of lactic acid in them during anoxemia need not necessarily be accompanied by an equal change in the hydrogen ion concentration of the blood. But in addition, increased pulmonary ventilation resulting from the increased acidity of the respiratory center washes out the acid of the blood and changes the balance of acid entering and leaving the blood to the alkaline side. Thus the lack of correspondence between the hydrogen ion concentration of the arterial blood and pulmonary ventilation is again explained.

The well-known increase in the amount of fixed acids in the blood, resulting from anoxemia, suggests that increased acidity of the tissues accompanies anoxemia. In this connection the experiments of Koehler, Brundquist and Loevenhart (106) are of particular interest. These investigators found in progressive anoxemia that a low oxygen tension of the respired air elicited hyperpnea, accompanied first by a decreased hydrogen ion concentration of the arterial blood, followed by an increase above normal. They believe that the production of fixed acids begins at once; that the increased pH of the blood is a result of increased elimination of carbon dioxide; that the final increased acidity of the arterial blood is due to failure of the increased elimination of acid to keep pace with the increased production of acid. These experiments support our contention of lack of correspondence between the pH of the blood and tissues; of lack of correspondence between the pH of arterial blood and pulmonary ventilation; and of the correspondence between the acidity of the respiratory center and pulmonary ventilation.

Returning to our hemorrhage experiments—what is the mechanism of the prompt apnea following injection of gum-saline solution during hyperpnea when the organism apparently is in need of oxygen? The increased flow of carbon dioxide carriers, the oxidation and removal of lactic acid, with the consequent reduction in the total amount of acid in the center, and the increased liberation of alkali in the blood seem adequate explanation. The same explanation is applicable to the apnea elicited by Eyster (107) by augmented flow of blood through a previously depressed respiratory center.

Apnea may also be elicited by abundant oxygen following anoxemia apparently independent of changes in the volume-flow of blood as illustrated by the administration of room air following hyperpnea from low oxygen tension in the respired air. Since these effects also occur within a few seconds the effects of oxygen on the acid metabolism of the respiratory center and on the dual function of hemoglobin appear sufficient in themselves to account for depressed respiration from abundant oxygen.

But in this connection our observations on hemorrhage require further elaboration. The period of apnea elicited by injection was frequently followed by a prolonged period of fifteen minutes or more of pulmonary ventilation considerably below normal, despite the dilution of the blood. Furthermore, the anomalous condition was noted that during this period of depressed ventilation, hyper-oxygen consumption considerably above normal obtained. Recovery consisted in a synchronous decrease of oxygen consumption and an increase in the pulmonary ventilation.

Similar hyper-oxygen consumption was noted in hemorrhage by Doi (108), in recovery from the administration of cyanide by Evans (109) and in unicellular organims by Lund (110). It most probably is similar to the hyper-oxygen consumption following muscular exercise dependent on the accelerated oxidation of accumulated lactic acid.

In that event the prolonged depression of respiration following the injection of gum-saline solution has a causal relation to the increased oxidations. It necessarily follows that the greater the oxidations, even though accompanied by a proportional increase in the formation of carbon dioxide, the greater becomes the alkalinity of the tissues. This is due not only to greater liberation of alkali in the tissues from altered acid metabolism, but to increased liberation of alkali in the surrounding blood as well, due to greater reduction of oxyhemoglobin.

Our view seems to be supported by a number of observations. Thus Koehler, Brundquist and Loevenhart find the abnormally acid blood of anoxemia turns more alkaline than normal during recovery. Henderson has noted beneficial effects of the administration of carbon diox-

ide along with oxygen in resuscitation from carbon monoxide poisoning. Though carbon monoxide anoxemia undoubtedly leads to the formation of abundant lactic acid, yet all this accumulated acid occurs in the form of sodium or potassium lactate. The displaced carbon dioxide is promptly eliminated. It is therefore suggested that the function of the administration of carbon dioxide is to satisfy the excessive alkali liberated in the blood and tissues by the increased removal of the lactate anion. At least the experiments of Barr and Himwitch (111) conform to this. They found that blood surcharged with lactic acid from exercise of one group of muscles when circulating through resting muscle suffered a loss of lactic acid accompanied by a decreased hydrogen ion concentration. Similar results have been obtained by Ronzoni (112).

From the foregoing it is obvious that during recovery from anoxemia the venous blood coming from the tissues as a whole may have a very low carbon dioxide tension. The general level of carbon dioxide tension of the blood must of necessity influence the effect of the acid metabolism of the respiratory center on its own activity. It is therefore essential to continually bear in mind that the respiratory center acts both as vanguard and rearguard to internal respiration.

VI. Miscellaneous remarks and applications. Since we have not as yet performed the crucial experiment of directly following the hydrogen ion concentration of the respiratory center, it is desirable to further test the application of our theory to respiratory phenomena and to consider possible objections to its acceptance.

It has frequently been stated that the latent period of hyperpnea elicited by anoxemia is too short to permit acid to accumulate in amount sufficient to stimulate the center (56), (10), (57), (50), (58), (59). The recent data on acid metabolism, the dual function of hemoblogin and the transport of acid, however, appear to meet this objection. Since reduced oxidations involve a decreased disappearance of the anaerobically formed lactic acid and decreased liberation of alkali by the hemoglobin, increased acidity and decreased oxidation in the tissues must of necessity be simultaneous processes occurring without an intervening latent period. That insufficient time elapses between anoxemia and hyperpnea for the accumulation of sufficient acid can hold only on the assumption that the respiratory center possesses a greater sensitivity to changes in oxygen tension than to changes in hydrogen ion concentration.

But if increased acidity invariably elicits reduced oxidation in the respiratory center the separation of the two factors becomes more complex. There are no data demonstrating a reduction in oxidation associated with the infinitely small changes in the pH of the blood

which elicit changes in ventilation yet in so far as strong mixtures of carbon dioxide reduce oxidation, weaker mixtures may also elicit reductions though infinitely small. In the absence of such data we have attempted to find instances of decreased pulmonary ventilation associated with reduced oxidation and exceptions to increased pulmonary ventilation and increased acidity of the respiratory center.

Every instance of increased pulmonary ventilation of chemical origin which we have found in the literature or in our work is associated with a theoretically increased acidity of the respiratory center. But the absence of respiration following forced ventilation even in the presence of grave cyanosis strongly suggests that reduced ventilation and reduced oxidation may go hand in hand, provided the hydrogen ion concentration of the respiratory center is below normal. Haldane and Poulton (44) found that the duration of apnea produced by forced ventilation varies with the excess of carbon dioxide washed from the system. They state

> When forced breathing is continued for a longer period, the apnea lasts much longer and the oxygen percentage in the alveolar air falls to such an extent that for a considerable time after the cessation of breathing the subject is blue in the face and presents a most alarming appearance although he feels no desire to breathe. The face gradually assumes a leaden corpse-like appearance characteristic of great anoxemia and it may be about a minute after this change begins before any desire to breathe is experienced. The result is in marked contrast to that observed when the oxygen percentage of the inspired air is allowed to fall pretty rapidly. In both cases increasing cyanosis is observed, but after the forced breathing there is neither hyperpnea nor any desire at all to breathe, until cyanosis is extreme, whereas, without forced breathing there is a marked hyperpnea as soon as cyanosis can be observed.

In keeping with these observations is the frequently expressed view that lack of oxygen exerts a paralyzing, rather than exciting action. Verworn (113) states that lack of oxygen has a general paralytic effect. Hill (73) finds that lack of oxygen stops the streaming of protoplasm. The unconsciousness from lack of oxygen described by Haldane and Poulton (44) and others is a well-known phenomenon. The fatigue of the respiratory center when resistance is offered to breathing is hastened by anoxemia (114). Eyster (107) found that the ligation of the cerebral arteries elicits apnea when the center is previously depressed by prolonged cerebral compression. Roberts (115) (116) has obtained comparable results. But, perhaps, more to the point are the findings of Mathison that when carbon dioxide fails to excite respiration lack of oxygen also fails but carbon dioxide may excite when lack of oxygen fails. To us it seems to follow that if the effects of carbon dioxide are due to reduced oxidation that the effects of lack of oxygen elicited by a direct method should be as great or greater. On the other

hand it is easy to understand why increased acidity from anoxemia should be without effect if simultaneous paralysis occurs.

Ignorant of the work of Mathison we reasoned that if the hyperpnea of lack of oxygen is an acid effect the effects of lack of oxygen and the administration of carbon dioxide should be additive. The additive effects of carbon dioxide and lack of oxygen from hemorrhage and of carbon dioxide and carbon monoxide have been described above. The effects of the administration of carbon dioxide with a low percentage of oxygen in the respired air are well known. We have also found that the hyperpnea elicited by sodium cyanide is increased still more by the administration of carbon dioxide. In this connection, though different, the additive effects of carbon dioxide and high oxygen tension might also be mentioned as another example of the addition of two acid effects. The fact that the central nervous system is very sensitive to increased acidity would accordingly explain its extreme sensitivity to both excess and lack of oxygen.

As regards the relative effects of oxygen and carbon dioxide in suspended breathing and in apnea the experiments of Werigo may be cited. Werigo (33) determined the effects of oxygen on the elimination of carbon dioxide by ventilating one lung with hydrogen and the other with oxygen and found that carbon dioxide in the lung ventilated with oxygen was invariably 2 to 3 per cent higher. He concluded that more than one half of the carbon dioxide formed in the body is driven out by the action of oxygen in the lungs. These experiments are a striking demonstration of the significance of the coördination of the dual function of hemoglobin in the transport and elimination of acid. They also emphasize the danger of drawing conclusions concerning the significance of alveolar carbon dioxide in the regulation of respiration.

Hill and Flack (117) state that "the inability to hold the breath depends more upon oxygen want than on carbon dioxide excess" and that "While Haldane and Priestley (26) are correct in saying that respiration is regulated by the tension of carbon dioxide in the alveolar air, in cases of obstructed aeration of the blood diminished oxygen tension is the more potent factor." Lutz and Schneider (118) state that "The earlier experiments did not distinguish clearly between the effects due to the lack of oxygen and those due to the accumulation of carbon dioxide. The complete separation of the two conditions by the use of such gases as hydrogen, nitrogen and carbon monoxide has proved that the initial effect of oxygen lack on the medullary center is clearly stimulating."

Yet the distinct separation of the effects of oxygen and carbon dioxide seems to be a very involved problem, for oxygen tension and rate of oxidation determine the kind and sum total of acid formed, the

transport of acid from and to the tissues and its elimination in the lungs. As a matter of fact, in the ultimate analysis it would appear that the rate of oxidation controls respiration by determining the efficiency of acid transport and the amount and kind of acid formed in the respiratory center and in the tissues as a whole.

It is a well-known fact that the administration of oxygen prolongs suspended respiration and increases the alveolar carbon dioxide at the breaking point. This has led to the view that oxygen enables the body to stand higher tensions of carbon dioxide (117). But there is another probability that increased oxygen tension increases the transport of carbon dioxide from the tissues to the lungs and drives the carbon dioxide from the blood into the alveoli of the lungs thereby protecting the body as a whole against high carbon dioxide tension. At least it is reasonably certain in Werigo's experiment that the lung possessing the higher alveolar carbon dioxide tension was the more efficient in protecting the body against the accumulation of carbon dioxide.

It has been suggested (119) that high arterial oxygen tension also protects the body against the bad effects of low carbon dioxide tension produced by forced ventilation, for forced ventilation with pure oxygen reduces or prevents the harmful effects of excessive ventilation. It seems, however, that these results may be explained by a hampered transport of carbon dioxide to the lungs and a reduced elimination there —due to a broken coördination of the dual function of hemoglobin. The fact that prolonged forced ventilation with pure oxygen shortens the subsequent period of apnea (119) is suggestive. While the beneficial effect of oxygen in the first instance is probably due to an improved transport of carbon dioxide, in the second instance it appears to be due to a hampered transport.

By special means Douglas and Haldane (38) varied the alveolar oxygen tension at the close of apnea elicited by forced ventilation and found that the alveolar carbon dioxide tension increased with an increase in the alveolar oxygen tension up to 120 mm. Hg tensions. At a tension of 120 mm. Hg the hemoglobin is virtually saturated with oxygen. On the other hand, higher oxygen tension decreases the alveolar carbon dioxide during normal respiration below the usual value (71), (75), (81). Bearing in mind the coördination of the dual function of hemoglobin, a maximum carbon dioxide tension should theoretically obtain when the hemoglobin is just completely saturated with oxygen. Either a lower or higher alveolar oxygen should reduce the alveolar carbon dioxide. These results consequently give further support to the significance of the dual function of hemoglobin in the regulation of respiration.

Periodic respiration elicited by a low percentage of oxygen in the respired air or by forced ventilation seems explicable by the indirect acid effects of lack of oxygen. During apnea anoxemia develops. Oxidations are reduced, accompanied by a decreased liberation of alkali by the hemoglobin and a hampered transport of acid. This coupled with an increased sum total of acid formed within the center through reduced oxidation elicits hyperpnea. During hyperpnea abundance of oxygen obtains. The excess of lactic acid which has accumulated elicits hyper-oxidation with a resultant diminution in the total amount of acid within the center. This is accompanied by a hyper-reduction of hemoglobin which increases the free alkali in the blood and improves the transport of acid from the center and thereby elicits apnea. The process is then repeated.

The frequent occurrence of periodic respiration with low alveolar oxygen tension is explainable by the shape of the oxygen dissociation curve of hemoglobin. The observation that periodic respiration may occur with a minimum alveolar oxygen tension of 120 to 130 mm. Hg; that the alveolar carbon dioxide tension may be increased by an increase in the normal alveolar oxygen tension agree with the extreme sensitivity of the respiratory center to changes in oxygen tension noted by Lutz and Schneider. These results suggest the indirect control of respiration by lactic acid.

Assuming that lactic acid is always present in the central nervous system and that the amount depends on the oxygen supply, a mechanism for the regulation of the effectiveness of the free carbonic acid by the determination of the value of the numerator and denominator of the carbonate buffer system obtains. Thus lactic acid assists in the control of respiration under so-called normal conditions as well as under conditions of stress.

## VII. SUMMARY AND CONCLUSIONS

I. Introduction. Theories on the chemical regulation of pulmonary ventilation are reviewed.

The general conception of the control of pulmonary ventilation by the composition of the blood includes as the primary *stimulating* factors the specific action of carbon dioxide, the hydrogen ion concentration and the oxygen tension of the arterial blood.

A critical analysis of the available data invariably establishes a lack of parallelism between pulmonary ventilation and any one of these factors.

In accordance with the theory proposed in this paper the activity of the respiratory center is fundamentally a function of its own acidity

as opposed to the acidity of the arterial blood, the specificity of carbon dioxide and the direct stimulating effect of lack of oxygen.

By virtue of its sensitivity to the hydrogen ion, the respiratory center responds to changes in its own acidity as influenced by its own acid metabolism, the acid metabolism of the tissues as a whole and associated changes in the transport of acid.

II. SPECIFIC ACTION OF CARBON DIOXIDE. As a possible objection to this theory, the specific action of carbon dioxide as a respiratory stimulant is analyzed.

Further evidence is presented indicating that carbon dioxide elicits acid effects by virtue of its properties of diffusion. By a simple diffusion experiment with the aid of color indicators it is demonstrated that an alkaline carbonate buffer solution of higher carbon dioxide tension may exert acid effects on a less alkaline buffer solution of lower carbon dioxide tension in the absence of a participating membrane.

The phenomenon is explainable by the fact that carbon dioxide diffuses more rapidly than sodium bicarbonate and so alters the $\frac{H_2CO_3}{NaHCO_3}$ ratio.

The presence of a differential barrier to the metallic cation in the living cell membrane should increase the acid effects of an alkaline carbonate buffer solution on the living cell still more.

The evidence and significance of such a barrier in relation to the regulation of respiration is discussed.

The fact that hyperpnea may occur with alkaline blood whether the carbon dioxide tension is considerably above or below normal seriously questions the specific action of carbon dioxide.

In explanation of the fact that the reduction of the blood pressure and respiration by excessive pulmonary ventilation is missing when the hydrogen ion concentration of the arterial blood is lowered an equal amount by the intravenous injection of sodium bicarbonate, it is suggested that the pH of the tissues is not equally altered under the two conditions and that such results are due to the differential diffusion of carbon dioxide and sodium bicarbonate between the tissues and the blood.

The intravenous injection of sodium bicarbonate not infrequently elicits hyperpnea in the presence of decreased hydrogen ion concentration of the arterial blood.

Since such injection is not associated with an increased carbon dioxide content of the arterial blood excluding the added sodium bicarbonate the specific action of the $HCO_3$ ion has been proposed.

Another explanation is offered as a substitute. Since the intravenous injection of sodium bicarbonate increases the freely diffusible

$CO_2$ and $H_2CO_3$ molecules at the expense of the less freely diffusible dissociated H and $HCO_3$ ions, and furthermore, since the rate of diffusion of the remaining dissociated acid is increased by the increase of the common $HCO_3$ ion, it is suggested that in the presence of a semipermeable membrane a double mechanism exists producing diffusion of carbon dioxide against a positive gradient.

Admitting this, it follows that the effects on the hydrogen ion concentration of the tissues produced by changes in the hydrogen ion concentration of the blood depend upon the method employed in varying the pH.

Whereas excessive elimination of carbon dioxide should elicit alkalemia, alkalosis and apnea, the intravenous injection of sodium bicarbonate might theoretically produce alkalemia, acidosis and hyperpnea. Experimental data support this view.

The available data indicate the probability of the explanation of the effects of carbon dioxide and of sodium bicarbonate on the basis of acidity.

III. THE HYDROGEN ION CONCENTRATION OF THE ARTERIAL BLOOD. The conception that the hydrogen ion concentration of the arterial blood regulates respiration is analyzed and found wanting.

Thus hyperpnea elicited by hemorrhage may be transformed into apnea by the intravenous injection of gum-saline solution.

Apnea so elicited comes on promptly as a result of an increased flow of unaltered blood through the respiratory center.

A fluctuation in pulmonary ventilation in the absence of changes in the hydrogen ion concentration of the arterial blood is, therefore, demonstrated.

Since the injection of gum-saline solution increases the nutrient flow, such apnea occurs in the presence of increased flow of free hydrogen ions to the respiratory center.

On the other hand, the increased flow of carbon dioxide carriers improves the transport of acid from the tissues.

If apnea is a function of the hydrogen ion it is, therefore, related either to an improved transport of acid from the center or to a disappearance of acid within the center.

Either of these conclusions necessarily involves the acid metabolism of the respiratory center as a factor controlling respiration.

Changes in the acid metabolism of the respiratory center by changing pulmonary ventilation elicit alterations in the pH of the arterial blood.

Thus the lack of correspondence between pulmonary ventilation and the hydrogen ion concentration of the arterial blood is in part explained.

Examples of such lack of correspondence are the hyperpnea of anoxemia, in which the blood is more alkaline than normal, and the apnea elicited by abundant oxygen following such hyperpnea which is associated with a sudden increased acidity of the arterial blood.

On the other hand the lack of correspondence between pulmonary ventilation and changes in the hydrogen ion concentration of the arterial blood elicited by the injection of sodium bicarbonate or of fixed acid is explainable by the differential diffusion of carbon dioxide and sodium bicarbonate.

Parallelism between pulmonary ventilation and arterial pH is an accidental occurrence prevailing only under special conditions.

IV. ARTERIAL OXYGEN AND THE DUAL FUNCTION OF HEMOGLOBIN. Theories on the mode of action of oxygen in regulating respiration are reviewed. Current theories on the action of lack of oxygen include stimulation by direct action, by the accumulation of acid, by an increase of excitability of the center or by the production of special metabolites which enter the blood stream and stimulate the center.

The harmful effects of hemorrhage and the beneficial effects of subsequent injection of a non-nutrient solution on the metabolism of tissues are reviewed in relation to the chemical control of respiration.

It is suggested that the harmful and beneficial effects respectively of hemorrhage and transfusion are probably due in the main to the accumulation of acid and lack of oxygen in the tissues consequent upon the altered transport of the blood gases.

In keeping with the impairment of the transport of carbon dioxide associated with a reduction in the volume flow of blood it was found that hemorrhage markedly increases the respiratory response to the administration of carbon dioxide in room air and also to the administration of room air alone while the subsequent injection of gum-saline solution reduces the ventilation.

Since the injection of gum saline solution dilutes the carbon dioxide carriers and buffers of the blood, the improved transport of carbon dioxide was attributed to the acceleration of the volume-flow of blood out of proportion to the dilution.

Since hemoglobin is almost equally responsible for the transport of oxygen and carbon dioxide, the conclusion that hyperpnea of hemorrhage is an acid effect rather than a direct effect of lack of oxygen is open to question.

Direct experiments show that exceedingly small hemorrhages elicit a decided reduction in the rate of oxygen consumption, which may be increased subsequently by the injection of gum-saline solution.

Therefore, the hemorrhage experiment, like a host of others, is a crucial test neither of the direct or of the indirect action of lack of

oxygen. Yet when considered in connection with the coördination of the dual function of hemoglobin and the transport of acid, the probability of the indirect action of lack of oxygen is markedly strengthened.

Under normal conditions the amount of acid formed in the tissues is approximately equal to the amount of alkali liberated in the blood and consequently the diffusion of carbon dioxide from the tissues remains undisturbed. But when oxidations are decreased while acids are being formed in unreduced amounts, the normal transport of acid from the center is impaired, due to a reduced liberation of the potential alkalinity of the blood as it passes through the center.

In the presence of lack of oxygen acid is, therefore, formed in greater abundance in the tissues than alkali is liberated in the blood.

This gives rise to a broken coördination of the dual function of hemoglobin.

The result is a decreased carbon dioxide diffusion gradient from the tissues to the blood and an increased acidity of both the tissues and the blood.

Since oxyhemoglobin, independent of any reduction, is a fairly efficient carbon dioxide carrier and buffer it may be objected that the hyperpnea of hemorrhage is due to a reduction in the flow of carriers rather than to a disturbance in the dual function of hemoglobin. But we found that the administration of carbon monoxide which increases the volume flow of blood increased the respiratory response to the administration of carbon dioxide and decreased rate of oxygen consumption. It is, therefore, inferred that reduced oxidation of hemoglobin in the lungs as well as reduced volume-flow of blood may elicit a disturbance in the coördination of the dual function of hemoglobin.

The hyperpnea elicited by sodium cyanide, which decreases oxidation independent of reduced volume flow or reduced arterial oxygen tension, is suggested as another example of disturbed coördination.

Since oxyhemoglobin cannot be reduced by the tissues so long as free oxygen is in solution in the plasma, it seems to follow that oxygen poisoning is due to the prevention of the liberation of the potential alkalinity of the blood. Unless the volume-flow of blood is enormously accelerated acidosis must obviously result. The hyperpnea of high oxygen tension, therefore, appears to be another example of the disturbed transport of acid from broken coördination of the dual function of hemoglobin.

Since oxygen poisoning is accompanied by decreased oxidation the question arises whether increased respiration results from decreased oxidation or increased acidity. Data are analyzed suggesting the latter.

If high oxygen tension produces acidosis it should increase the toxicity of carbon dioxide. Experiments on rats show that whereas a pres-

sure of 3.5 atmospheres of oxygen, or a 5 per cent mixture of carbon dioxide increases respiration, the combination of 5 per cent carbon dioxide with 3.5 atmospheres of oxygen elicits marked dyspnea. Whereas, approximately 3.5 atmospheres of oxygen or 5 per cent mixture of carbon dioxide are compatible with life for several hours, the combined effects produce rapid dissolution.

It is concluded that the dual function of hemoglobin is very important in the transport of acid and the regulation of respiration; that a broken coordination may occur with a reduced volume-flow of blood, with reduced oxidation of the hemoglobin in the lungs, with low percentage of hemoglobin, with reduced oxidations independent of oxygen supply as in cyanide poisoning, and under the peculiar condition of oxygen poisoning where the abundance of oxygen in the plasma prevents a reduction of oxyhemoglobin in the tissues and oxidation in the lungs.

V. LACTIC ACID AND THE ACID METABOLISM OF THE RESPIRATORY CENTER. Data on the significance of lactic acid and the acid metabolism of the respiratory center are reviewed. They appear to justify the assumption that the acid metabolism of the respiratory center responds like striated muscle to oxygen supply and to explain the effects of oxygen on pulmonary ventilation.

Since only 3 molecules of carbon dioxide are formed for every 4, 5 or 6 molecules of lactic acid which disappear in the presence of oxygen it follows that for every 4, 5 or 6 molecules of lactic acid failing to disappear during anoxemia the amount of acid formed per unit reaction is increased 33, 66 or 100 per cent and conversely, for every 4, 5 or 6 molecules of lactic acid which disappear there is a corresponding decrease in the amount of acid.

Since lactic acid reacts with sodium bicarbonate, changes in the acid metabolism effect the numerator and denominator of the carbonate buffer system $\left(\frac{H_2CO_3}{NaHCO_3}\right)$ Lack of oxygen increases the numerator and decreases the denominator with a consequent increase in acidity while a subsequent abundance of oxygen increases the denominator and decreases the numerator with a consequent increased alkalinity.

Independent of changes in the transport of acid such alterations in the acid metabolism of the respiratory center might of themselves explain the sensitiveness of the center to changes in oxygen supply.

Lack of oxidations, however, involves a decreased liberation of alkali in the blood and thereby reduces the carbon dioxide diffusion gradient from the center to the blood.

A double mechanism, therefore, obtains for the accumulation of acid in the tissues during anoxemia and explains the hyperpnea of hemorrhage and of lack of oxygen in the respired air.

Similarly with a subsequent abundance of oxygen, oxidations return; increased liberation of alkali in the blood stream occurs synchronous with a liberation of alkali within the tissues.

Assuming that the major portion of the carbon dioxide displaced from the sodium bicarbonate by lactic acid in the respiratory center and in the tissues as a whole has been eliminated, the synchronous liberation of alkali in the center and the blood amply explains the apnea following injection of gum-saline solution. The apnea on the administration of room air following hyperpnea from lack of oxygen is similarly explained.

But apnea produced by the injection of gum-saline solution may be followed by a prolonged period of pulmonary ventilation considerably less than normal.

This depressed ventilation is accompanied by a marked hyper-oxygen consumption. Evidence indicates that this hyper-oxygen consumption represents excessive oxidation of accumulated lactic acid. An anomalous condition, therefore, arises. Despite the increased production of carbon dioxide, the greater the oxygen consumption, the greater is the increased alkalinity developed within the tissues and in the blood. The prolonged period of depressed respiration during recovery from anoxemia is thus explained.

It is suggested that the phenomenon underlying this depressed respiration explains the beneficial effects of the simultaneous administration of carbon dioxide and oxygen in the resuscitation from carbon monoxide poisoning. The observation that the hydrogen ion concentration of blood containing excess of lactic acid is decreased on passing through resting muscle and that recovery from general anoxemia is accompanied by an increased alkalinity of the blood greater than normal, supports this suggestion.

It is concluded that lactic acid is an important regulator of respiration, exerting its effects under normal conditions as well as under conditions of stress, by varying the numerator and denominator of the carbonate buffer system, thereby governing the effectiveness of the free carbonic acid.

VI. Miscellaneous remarks and applications. Possible objections to our views are considered and further miscellaneous data are reviewed.

In conclusion the following points are emphasized:

Changes in acidity of the respiratory center constitute the primary chemical control of respiration.

Changes in oxidation constitute a less direct control. But insofar as oxidation determines the kind and amount of acid formed and the efficiency of their transport and elimination, it is in the end the normal regulator of respiration.

In accordance with this view two kinds of acid make up the normal respiratory stimulus—a poorly diffusible and fixed acid, such as lactic acid, and the weak, volatile and readily diffusible carbon dioxide, the respiratory stimulus being the sum of the effects of both.

During anoxemia reduced oxidations in the center leads to an increased formation of acid and to an accumulation of the relatively poorly diffusible acid in a relatively poorly buffered cytoplasm no longer fully protected by the potential alkalinity of the blood or by its own buffer salts. By virtue of its own acid metabolism and its extreme sensitivity to minute changes in its own pH the center is sensitive to minute changes in the oxygen tension of the arterial blood and is in a position to act as vanguard to internal respiration.

The capacity of the center to act as rearguard, however, must not be overlooked. Since fluctuations in the general level of carbon dioxide and oxygen in the blood is a function of the fluctuations in the general metabolism as well as of pulmonary ventilation, the respiratory center must of necessity continually function both as vanguard and rearguard to internal respiration.

## BIBLIOGRAPHY

(1) Haldane: Respiration. New Haven, 1922.
(2) Haggard and Henderson: Journ. Biol. Chem., 1920, xliii, 3.
(3) Haldane, Kellas and Kennaway: Journ. Physiol., 1919–20, liii, 180.
(4) Scott: This Journal, 1918, xlvii, 43.
(5) Haldane: The organism and environment. New Haven, 1917.
(6) Cohen: Journ. Pharm. Exper. Therap., 1918, xi, 221.
(7) Winterstein: Arch. f. d. gesammt. Physiol., 1911, cxxxviii, 168.
(8) Laquer and Verzar: Arch. f. d. gesammt. Physiol., 1912, cxliii, 395.
(9) Hasselbalch: Biochem. Zeitschr., 1912, xlvi, 416.
(10) Hooker, Wilson and Connett: This Journal, 1917, xliii, 351.
(11) Jacobs: This Journal, 1920, li, 321.
(12) Jacobs: This Journal, 1920, li, 457.
(13) Collip: Journ. Physiol., 1920–21, liv, 58.
(14) Dale and Evans: Journ. Physiol., 1922, lvi, 125.
(15) Bayliss: Principles of general physiology. New York, 1915, 142.
(16) Parsons and Shearer: Journ. Physiol., 1920–21, liv, 62.
(17) Michaelis: Die Wasserstoffionenkonzentration. Berlin, 1922.
(18) Gesell: Proc. Soc. Exper. Biol. and Med., 1922, xx, 174.
(19) Fletcher: Journ. Physiol., 1898–99, xxiii, 10.
(20) Loeb: Studies in general physiology. Chicago University Press, 1913.
(21) Loeb: Journ. Gen. Physiol., 1922, v, 255.
(22) Arrhenius: Theories of solution. New Haven, 1912, 163.
(23) Loeb: Journ. Gen. Physiol., 1922, v, 255.
(24) Sizili: Arch. f. d. gesammt. Physiol., 1909, cxxx, 134.
(25) Fitzgerald: Phil. Trans. Roy. Soc. of London, 1912–13, cciii, Ser. B, 350.
(26) Haldane and Priestley: Journ. Physiol., 1905, xxxii, 225.
(27) Campbell, Douglas and Haldane: Journ. Physiol., 1914, xlviii, 303.

(28) CAMPBELL, DOUGLAS, HOBSON AND HALDANE: Journ. Physiol., 1913, xlvi, 301.
(29) LOEWY: Arch. f. d. gesammt. Physiol., 1899, xlvii, 601.
(30) GESELL, CAPP AND FOOTE: This Journal, 1922, lxiii, 1.
(31) PARSONS: Journ. Physiol., 1917, li, 440.
(32) CHRISTIANSEN, DOUGLAS AND HALDANE: Journ. Physiol., 1914, xlv, 244.
(33) WERIGO: Arch. f. d. gesammt. Physiol., 1892, li, 321.
(34) BARR AND PETERS: Journ. Biol. Chem., 1921, xlv, 57.
(35) FRASER, ROSE AND DREYER: Quart. Journ. Med., 1922, xv, 195.
(36) MELLANBY: Journ. Physiol., 1922, lvi, Proceedings, 38.
(37) GESELL: This Journal, 1919, xlvii, 428.
(38) DOUGLAS AND HALDANE: Journ. Physiol., 1908, xxxvii, 355.
(39) DOUGLAS AND HALDANE: Journ. Physiol., 1909, xxxviii, 420.
(40) DOUGLAS: Journ. Physiol., 1910, xl, 454.
(41) MATHISON: Journ. Physiol., 1910, xli, 416.
(42) MATHISON: Journ. Physiol., 1911, xlii, 283.
(43) BOYCOTT AND HALDANE: Journ. Physiol., 1908, xxxvii, 355.
(44) HALDANE AND POULTON: Journ. Physiol., 1908, xxxvii, 390.
(45) GEPPART AND ZUNTZ: Arch. f. d. gesammt. Physiol., 1882, xlii, 189.
(46) DOUGLAS, HALDANE, HENDERSON AND SCHNEIDER: Phil. Trans. Roy. Soc. of London, Series B, 1912–13, cciii, 185.
(47) PFLÜGER: Arch. f. d. gesammt. Physiol., 1868, i, 61.
(48) LINHARD: Journ. Physiol., 1911, xlii, 336.
(49) HASSELBALCH: Biochem. Zeitschr., 1912, xxxviii, 77.
(50) MILROY: Quart. Journ. Exper. Physiol., 1914, viii, 141.
(51) ROSENTHAL: Herman's Handbuch der Physiologie, Bd. iv, 269.
(52) HEAD: Journ. Physiol. 1889, x, 1.
(53) GREGG, LUTZ AND SCHNEIDER: This Journal, 1919–20, l, 302.
(54) ELLISS: This Journal, 1919–20, l, 228.
(55) LUTZ AND SCHNEIDER: This Journal, 1919–20, l, 228.
(56) SCOTT: Journ. Physiol., 1908, xxxvii, 301.
(57) BEDDARD, PEMBREY AND SPRIGGS: Journ. Physiol., 1908, xxxvii, p. xl.
(58) GASSER AND LOEVENHART: Journ. Pharm. Exper. Therap., 1914, v, 239.
(59) LUTZ AND SCHNEIDER: This Journal, 1919–20, l, 327.
(60) GESELL: This Journal, 1919, xlvii, 428.
(61) GESELL: This Journal, 1919, xlvii, 438.
(62) GESELL: This Journal, 1919, xlvii, 468.
(63) GESELL: This Journal, 1920, liv, 166.
(64) GESELL, BLAIR AND TROTTER: This Journal, 1922, lxi, 399.
(65) GESELL AND MOYLE: This Journal, 1922, lxi, 412.
(66) GESELL AND MOYLE: This Journal, 1922, lxi, 420.
(67) GESELL, CAPP AND FOOTE: This Journal, 1922, lxiii, 32.
(68) GESELL, FOOTE AND CAPP: Proc. Soc. Exper. Biol. and Med., 1922, xx, 174.
(69) VAN SLYKE: Physiol. Rev., 1921, i, 141.
(70) HENDERSON: Journ. Biol. Chem., 1920, xli, 401.
(71) BERT: La Pression Barométrique. Paris, 1878.
(72) SMITH: Journ. Physiol., 1899, xxiv, 19.
(73) HILL: Recent advances in physiology and biochemistry. London, 1908, 196.
(74) HILL: Caisson sickness. London, 1912.
(75) HILL AND MACLEOD: Journ. Physiol., 1903, xxix, 382.
(76) HILL AND MACLEOD: Journ. Physiol., 1903, xxix, 492.
(77) PANUM: Arch. f. d. gesammt. Physiol., 1868, i, 125.

(78) DAUTREBANDE AND HALDANE: Journ. Physiol., 1921, lv, 296.
(79) BURROWS: This Journal, 1917, xliii, 13.
(80) PARKINSON: Journ. Physiol., 1912, xliv, 54.
(81) YAMADA: Biochem. Zeitschr., 1918, lxxxix, 27.
(82) SNELL: Compressed air illness. London, 1896.
(83) FLETCHER: Journ. Physiol., 1898–99, xxiii, 10.
(84) FLETCHER: Journ. Physiol., 1902, xxviii, 354.
(85) FLETCHER: Journ. Physiol., 1902, xxviii, 474.
(86) FLETCHER AND HOPKINS: Journ. Physiol., 1907, xxxv, 247.
(87) HILL: Physiol. Rev., 1922, ii, 310.
(88) MEYERHOF: Arch. f. d. gesammt. Physiol., 1921, clxxxviii, 114.
(89) MEYERHOF: Arch. f. d. gesammt. Physiol., 1922, cxcv, 22.
(90) VERZAR: Journ. Physiol., 1912–13, xlv, 139.
(91) KROGH: Journ. Physiol., 1919, lii, 457.
(92) SPIRO: Zeitschr. f. Physiol. Chem., 1877–78, i, 111.
(93) ARAKI: Zeitschr. f. Physiol. Chem., 1891, xv, 335.
(94) ARAKI: Zeitschr. f. Physiol. Chem., 1891, xv, 546.
(95) ZILLESSEN: Zeitschr. f. Physiol. Chem., 1891, xv, 387.
(96) ARAKI: Zeitschr. f. Physiol. Chem., 1892, xvii, 311.
(97) ARAKI: Zeitschr. f. Physiol. Chem., 1894, xix, 422.
(98) RYFFEL: Zeitschr. f. Physiol. Chem., 1909, xxxix, 5.
(99) MACLEOD: This Journal, 1920, lv, 184.
(100) FRIES: Biochem. Zeitschr., 1911, xxxv, 368.
(101) CLAUSEN: Unpublished work.
(102) LANGENDORFF: Biol. Centralbl., 1886–87, vi, 188.
(103) MANDEL AND LUSK: This Journal, 1906, xvi, 129.
(104) LUSK: Harvey Lecture, 1908–09.
(105) DAKIN: Physiol. Rev., 1921, i, 394.
(106) KOEHLER, BRUNQUIST AND LOEVENHART: This Journal, 1923, lxiii, 404.
(107) EYSTER: Journ. Exper. Med., 1906, viii, 565.
(108) DOI: Journ. Physiol., 1921, lv, 249.
(109) EVANS: Journ. Physiol., 1919–20, liii, 17.
(110) LUND: Biol. Bull., 1921, xli, 203.
(111) BARR AND HIMWICH: Journ. Biol. Chem., 1923, lv, 525.
(112) RONZONI: Unpublished work.
(113) VERWORN: Arch. f. Physiol., 1903, lxv.
(114) HALDANE, MEAKINS AND PRIESTLEY: Journ. Physiol., 1919, lii, 420.
(115) ROBERTS: Journ. Physiol., 1921, lv, 346.
(116) ROBERTS: Journ. Physiol., 1921, lvi, 101.
(117) HILL AND FLACK: Journ. Physiol., 1908, xxxvii, 77.
(118) LUTZ AND SNYDER: This Journal, 1919–20, l, 280.
(119) HILL AND FLACK: Journ. Physiol., 1910, xl, 347.

# FURTHER EXPERIMENTS ON THE SENSITIZATION TO HEAT DUE TO EXPOSURE OF SHORT WAVE-LENGTHS

## Influence of Ozone

H. S. FORBES and G. A. DALAND

*From the Laboratories of Biophysics, Harvard University, Boston*

Received for publication May 26, 1923

It has been shown by Bovie (1) that ultra-violet light will cause such a change in egg albumen that when heated it will coagulate at a much lower temperature than before. Preliminary experiments by Bovie and Klein indicated that ultra-violet light produced a similar effect upon the living organisms (Paramecia) (2). The following experiments were undertaken with the view of extending these observations and of controlling some of the conditions more accurately.

*Method.* The source of ultra-violet light, a small hydrogen lamp with a fluorite window at the top, has been described (3). A pedigreed stock culture of Paramecium caudatum was used which had been dividing regularly for over two months. The medium was hay infusion with occasional brief substitution of beef extract.

The technic used in exposing Paramecia to the light was as follows: The Paramecia were transferred by a glass pipette from the stock solution to four drops of fresh hay infusion on a clean slide. One organism was then drawn into the tube of a red-blood-cell-counter pipette and expelled with a tiny drop of fluid upon the fluorite slide. This was a glass microscope slide in which a hole had been bored and a disk of fluorite 15 mm. in diameter inserted and held by De Khotinsky cement. The fluorite disk was sunk so that its upper surface was 0.25 mm. below the upper surface of the glass microscope slide. A large heavy cover glass was placed over this depression, thus forming an "exposing chamber" 0.25 mm. in depth. The drops in which the organisms were exposed were always large enough to wet the underside of the cover glass. The drops, therefore, had a uniform depth. The diameter of the drop was measured and kept constant to within 0.5 mm., and in these experiments was not large enough to completely fill the depression chamber. The drop was, therefore, in contact with the air enclosed within the chamber.

The fluorite slide was placed upon the fluorite window of the lamp and exposure made for a given number of seconds by pressing a key which closed the electric circuit.

In some experiments the radiated organisms were subjected to heat. For this heating an electric warm stage was used which had a strip of copper in the bottom to distribute the heat more evenly.

The Paramecia were transferred by pipette from the fluorite slide to 4 or 5 drops of hay infusion in the concavity of a glass slide which was placed in the warm stage chamber and the whole was covered with a glass plate. The temperature was measured by a thermocouple, the variable junction of which was kept in the culture drop throughout the period of heating. The temperature was maintained within one degree of 34°C. for five minutes. The optimum temperature for Paramecia is 24° to 28°C. (5), and we found 38° to 40° to be lethal. After treatment the Paramecia were transferred to drops of hay infusion on depression slides and the next day the record of lethal effects was taken.

Five series of experiments were conducted. In the first series the organisms were radiated first and then heated for five minutes. In a second series the organisms were heated first and then radiated. The other three series were controls; radiation without heat, heat without radiation, and the effects of manipulation.

*Discussion.* In a series of over 200 experiments the results showed that the number of deaths in the first series, that is, the series in which the heat followed the radiation, was not greater than in any of the other four series.

Two factors were noticed to have an important influence upon the death rate. The first was the size of the drop; the smaller the drop the more lethal the effect. This had been observed by Bovie and Klein but these investigators did not conduct successful experiments to determine its cause. In their experiments they were content to keep all of the drops in which the organisms were exposed of the same size. The second factor was the length of time the cover glass remained on the exposing chamber after the radiation. It was found that death always followed if the cover glass was kept on for some time.

A search for the cause of these two variations was made. It had been observed that immediately after using the lamp a definite odor of ozone was noticeable above the fluorite window, and Lyman (6) had shown that ozone is formed rapidly in that region of the spectrum where oxygen has a strong absorption band (1850 to 1250 Å). In our work the depth of the chamber was so small (0.25 mm.) and the length of the exposure to the rays was so short that we had assumed that the amount of oxygen present and, accordingly, the amount of ozone

formed, was negligible. When it was found that a toxic substance was formed, the following experiments were conducted in order to determine whether or not ozone was formed in the fluorite chamber and in amount sufficient to be toxic to the Paramecia.

The fluorite slide which was used in the above experiments was modified so that a second similar depression chamber was formed on the same microscope slide. This second chamber, however, had a glass bottom in place of the fluorite disk. These two chambers were connected by a tubular channel formed in wax. The needle of a hypodermic syringe was connected through a wax seal to the second chamber in such a manner that the air contained in the first (fluorite) chamber could be drawn over into the second chamber. A small drop of hay infusion containing the Paramecia was placed in the second chamber. The fluorite chamber was radiated for twenty seconds and the radiated air was then drawn over into the second chamber. The Paramecia in the second chamber were killed. The control experiment in which unexposed air was drawn through the two chambers produced no harmful effect on the Paramecia. Clearly some diffusible substance, toxic to Paramecia, was formed in the fluorite chamber during radiation. Experiments were, therefore, made to determine the nature of this toxic substance. The following substances were placed in the fluorite chamber and so disposed about the edges of the chamber that they did not lie in the beam of the fluorite rays. Any effects obserevd, therefore, would not be due to the direct action of the radiation.

*a.* A small bit of filter paper moistened with potassium-iodide-starch solution.

*b.* A small drop of potassium-iodide-starch solution.

*c.* A small drop of hay infusion containing three Paramecia.

The cover-glass was placed over all and kept on during the observation. The chamber was radiated 16 seconds.

*Results: a.* The moist filter paper turned blue at once.

*b.* The potassium iodide starch drop gradually turned blue, starting at the edge of the drop nearest the radiated portion of the chamber.

*c.* The Paramecia began to twitch about the same moment the color change appeared in the potassium-iodide-starch drop. Cytolysis of all Paramecia occurred within one minute.

The toxic substance was evidently either ozone or hydrogen peroxide (potassium-iodide-starch test). It proved to be ozone diffusing into the drops and not hydrogen peroxide formed directly within the drop, for when the chamber was filled with potassium-iodide-starch solution so that there was no air present, no color change occurred with an exposure of 20 seconds. If minute air bubbles remained in the chamber during radiation a local blueing of the starch grains occurred about them.

The literature up to the early part of the twentieth century contains many contradictory statements in regard to the mode of action of light.

In 1905 Valdemar Bie (7) of the Finsen Institute, collected the data in the literature and repeated many of the experiments of the early investigators. Bie has shown that:

1. The effect on the organism is due directly to the action of the light and not to the formation of toxic substances in the medium.

2. Light kills when there is no free oxygen present and when there is no means by which any may be formed.

3. Light of short wave-length kills regardless of the amount of oxygen.

4. The presence of oxygen is more important with light of longer wave-lengths.

Later investigators, Henri (8) and Carl (9), apparently independently, came to the same conclusion as Bie, namely, that the action of light is a direct action on the organism and that the abiotic action of light increases as the wave-length decreases.

With ultra-violet light of short wave-lengths (1850 to 1250 Å.) Bovie (10) has found that it requires as long to kill spores of Monilia sp., in the presence of oxygen (air) at atmospheric pressure, as it does in a very high vacuum.

Bovie (11) has shown that Paramecia may be killed by the direct action of fluorite rays upon the organism without the action of intermediary photoproducts being formed from the culture solution.

These experiments of Bovie's were repeated. Paramecia were exposed one at a time to the direct ultra-violet rays to determine the destructive action of the rays when ozone was excluded. Ozone formation was prevented by filling the chamber with hay infusion without bubbles. The observed effects were as follows:

The Paramecia directly in the path of the rays were completely cytolyzed in 16 to 20 seconds. Those organisms outside of the beam of fluorite rays showed no injurious effects. When these unexposed organisms passed through the exposed area immediately after the light was turned off they showed no reaction which would indicate that the radiated culture solution contained a toxic substance. Further, these non-exposed organisms showed no signs of injury when they were kept in the exposing chamber for half an hour after radiation.

It seemed probable, therefore, that the effects observed by Bovie and Klein were not the result of heat sensitization, but were spurious effects due to ozone formed by the fluorite rays. It now remained to exclude the ozone and to determine whether or not Paramecia exposed to fluorite radiation are sensitized to the influence of heat. A short

series of experiments was carried out using more intense radiation and observing the organisms during both radiation and heating until cytolysis had occurred. This series showed a much greater lethal effect upon the Paramecia if they were radiated first and heated afterwards than if the procedure was reversed. These positive results led to a decision to undertake an extensive series of experiments using different ranges of temperatures.

## CONCLUSIONS

Paramecium caudatum exposed to ultra-violet light in a fluorite chamber may be killed either by the direct action of the rays or by the ozone formed.

Ozone is formed by the action of ultra-violet rays upon air under the conditions of these experiments. It is rapidly absorbed by the culture fluid and then exerts an intensely toxic action on the contained organisms.

Exclusion of air bubbles prevents ozone formation. The direct action of the rays can then be proved lethal.

Heat following strong radiation has apparently a more lethal effect than preceding it. Further experimental evidence than that presented in this paper has confirmed this finding.

## BIBLIOGRAPHY

(1) Bovie: Science, 1913, xxvii, 373.
(2) Bovie and Klein: Journ. Gen. Physiol., 1919, i, 331.
(3) Bovie: Bot. Gazette, 1916, lxi, 1.
(4) Woodruff and Baitsell: Journ. Exper. Zoöl., 1911, xi.
(5) Woodruff and Baitsell: This Journal, 1911–12, xxix, 147.
(6) Lyman: Astrophys. Journ., 1908, xxvii.
(7) Bie: Mit. Finsens Med. Lysinstitut, 1905, ix, 75; Ibid., 1905, ix, 5.
(8) Henri: Mém. Soc. Biol., 1912, lxxiii, 323.
(9) Carl: Beitr. Biol. Pflanzen, 1915, xii, 435.
(10) Bovie: Unpublished experiments.
(11) Bovie: The abiotic action of fluorite rays. A thesis on file in the Widener Library, Harvard University.

# NEW EXPERIMENTS ON THE SENSITIZATION OF PROTOPLASM TO HEAT BY EXPOSURE TO LIGHT OF SHORT WAVE-LENGTH

W. T. BOVIE AND G. A. DALAND

*From the Laboratories of Biophysics, Harvard University, Boston*

Received for publication May 26, 1923

It has been shown in the preceding paper (1) that the heat sensitization experiments reported by Bovie and Klein (2) were invalidated by reason of the spurious effects of the ozone generated by the fluorite rays to which the organisms were exposed. This paper is a report of new experiments on the influence of heat upon *Paramecium caudatum* after the organism has been exposed to fluorite rays. We believe that the influence of ozone has been excluded. The results of these new experiments lead to the same conclusion put forward in the former paper, namely, that by an exposure to fluorite rays the organism is rendered extremely sensitive to heat, so that it is injured and killed by an amount of heat which would not affect a normal unradiated individual.

The results of these experiments are important: first, because of the insight they give us concerning the nature of the destructive action of radiation; second, the important bearing they may have upon considerations of the dosage of radiation; and third, the possible application to new methods in ray therapy.

The first visible effect of fluorite rays upon Paramecium is stimulation. An exposure of a fraction of a second is sufficient to stimulate a dormant organism to activity. An exposure of a few seconds causes injuries, the extent of which are dependent upon the intensity of the radiation, upon the length of the exposure, and upon "individual idiosyncrasies" of the organism.

The typical visible effects observed in an organism during an exposure to fluorite rays of sufficient intensity are, first, an increase in the rate of locomotion ("stimulation") followed by the "avoiding reaction" which is characteristic of Paramecium. The organism then moves around in an irregular, or more or less circular path, its rate of locomotion decreasing until it finally ceases altogether. The body becomes short, thickened, and often bent. On that part of the organism receiving the radiation directly, the cilia may cease to beat, but on the

other parts of the organism, particularly within the gullet, the beating of the cilia continues. Vesicles form on some of the organisms. Sometimes these vesicles rupture and the disorganizing protoplasm streams out into the surrounding medium. Other organisms disorganize internally, parts of the protoplasm aggregating into small isolated spheres. There is usually an increase in the size of the vacuoles in the early stages of this fluorite-ray cytolysis. Circulation of the food vacuoles was observed in normal Paramecia, and in Paramecia which had recovered from the effects of radiation, but it was not observed in organisms showing severe effects of radiation.

For the experiments shown in this paper, the intensity of the fluorite radiation was such that complete cytolysis required an exposure of about forty-five seconds. This was the average exposure required for complete cytolysis when the organisms received continuous radiation. With shorter exposures, the cytolytic changes were less intense and the time of their appearance was delayed. It was possible to adjust the length of exposure so that complete cytolysis occurred in but a small per cent of the radiated organisms. The majority of these recovered after passing through the first stages of cytolysis.

The process of recovery was slow. Circulation of the food vacuoles was renewed, the beating of the cilia became more vigorous and the organism began to move slowly, usually in a circle. It slowly rotated on its long axis as it continued on its circular path, the movements gradually becoming more and more normal. Finally the path straightened out until it was impossible to distinguish the movements of the recovered radiated organism from those of a non-radiated organism. In a few cases recovery was observed in organisms which had formed vesicles during the radiation. Recovery after the appearance of vesicles was, however, comparatively rare.

The time elapsing between the end of the exposure and the appearance of any delayed cytolytic change is referred to in this paper as the *latent period*. It is the object of this investigation to study the influence of heat upon the duration of this latent period.

As in previous experiments, the organisms were cultured in drops of hay infusion on microscope slides. From time to time, when the organisms appeared to be in periods of metabolic depression, small amounts of beef extract were added to the hay infusion (3). In these new experiments an isolated Paramecium was allowed to multiply until the drop contained four organisms. Three organisms were used in each experiment as described below. The fourth was transferred to a fresh drop in which it multiplied to form four organisms for the next experiment. In this way all of the organisms used in any one experiment were descended from a single individual.

The source of radiation was the hydrogen discharge tube which has been described in previous papers (4). The discharge tube is so constructed that it fits under the microscope stage in the place of the usual sub-stage accessories. The tube was adjusted so that its fluorite window was flush with the top of the microscope stage. The current for exciting the tube was drawn from the secondary circuit of a one-half kilowatt transformer (Clapp-Eastham). In these experiments, the exciting current was controlled by varying the voltage of the current in the primary circuit of the transformer. In the secondary circuit of the transformer, in series with the discharge tube, there was placed a small coil (about 3 mm. in diameter) consisting of several turns of "Advance" wire. The "Advance" wire was one mill in diameter and its temperature was raised appreciably by the current (10 to 20 milliamperes) passing through the discharge tube. The variable junction of a copper-constantan thermocouple was supported in the center of this coil and the whole enclosed in an air-tight metal case. The leads of the thermocouple were connected through a double-pole, double-throw switch with a sensitive low resistance galvanometer (Leeds and Northrup Co., no. 2500-A). The galvanometer deflections were, therefore, correlated with the amount of current flowing through the discharge tube. The voltage in the primary circuit of the transformer was adjusted so as to maintain the galvanometer deflections reasonably constant. This did not, of course, insure that the intensity of the fluorite radiation emitted by the discharge tube was always constant. It probably was not as constant as the galvanometer deflections, for the aluminum electrodes within the discharge tube contain absorbed gases, which are driven out when the tube is excited. Hence the gas pressure within, and therefore the resistance of the discharge tube, was not constant. The proportion of the energy of the exciting current dissipated in the form of heat would probably vary with the electrical resistance of the tube. No convenient method of directly measuring the intensity of the fluorite radiation is available. The precision of this indirect method of controlling the intensity of the radiation will find expression in the constancy of the effects of the rays upon the organisms and will be summed up along with the variations charged to "individual idiosyncrasies of susceptibility."

The three organisms to be used in a single experiment were transferred to a small drop of hay infusion on a disc of fluorite. The fluorite disc was held in the steel frame described below.

The steel frame was 7.5 cm. long and 2.5 cm. wide, and was given plane parallel surfaces by passing it repeatedly through a planer and then scraping with a hand tool until the surface formed a continuous contact when tested with a standard flat surface.

A hole 1.5 cm. in diameter was bored through it, and within this hole the fluorite disc was held with its lower surface flush with the lower surface of the frame. The steel frame was 100 $\mu$ thicker than the fluorite disc—the disc and the frame forming a *depression slide* with a flat bottomed depression just 100 microns deep.

The organisms to be exposed were placed in a drop of culture medium upon the fluorite disc and a cover glass placed over the top. In order to exclude the effects of ozone, care was taken to use a sufficiently large drop of culture medium so that when the cover glass was placed on the depression slide no air bubbles were enclosed. Air was further excluded by sealing the edge of the cover glass to the steel frame with vaseline.

The culture media must have been quite opaque to the fluorite rays and undoubtedly protected the organisms from those rays which are most readily absorbed, that is, those having the shortest wave-length (5). In some of the preliminary experiments the organisms were exposed in tap water, which is undoubtedly more transparent to fluorite rays than culture media, but we found that undesirable complications were introduced by the toxic effects of the tap water.

The fluorite depression slide containing the three organisms was placed on the stage of the microscope with the fluorite disc in direct contact with the fluorite window of the discharge tube. Observing the organisms with the binocular microscope, the frame was moved about until one was directly over that part of the fluorite window of the discharge tube through which the fluorite rays were emitted. A key in the primary circuit of the transformer was closed and the exposure made. The steel frame was manipulated during the exposure so as to keep the single organism in the beam of fluorite rays. If one of the other two organisms was accidentally exposed the experiment was rejected.

The fluorite depression slide containing the one exposed organism and two unexposed organisms was then placed in an electrically heated incubator for use on the microscope stage. The temperature of the incubator was automatically controlled by a thermostat but when necessary the heating current was controlled by hand.

The temperature to which the organisms were heated was measured by a copper-constantan thermocouple which could be connected with the same galvanometer used in regulating the current through the discharge-tube, through the double-pole, double-throw switch mentioned above.

It was impracticable to place the variable junction of the thermocouple directly in the drop containing the organism because of difficulties in keeping the depression chamber air-tight. Therefore the variable junction was soldered to the metal frame as close as possible to the depression chamber.

Experiments were conducted to test the precision of this method of measuring the temperature to which the organisms were heated. Measurements were made both while the incubator was warming up and cooling down. It was found that the difference in temperature registered by the junction in the drop and one soldered on the slide was never greater than 0.2 degree.

The time required for the depression slide to warm up to the incubator temperature was shortened by providing the incubator with a heavy brass floor upon which the depression slide rested. The floor served as a heat reservoir. It contained a round glass window for transmitting light from the microscope mirror. Under the best conditions the organisms reached the desired temperature about two minutes after being placed on the warm stage and could then be maintained within 0.8°C. of this temperature.

The method of conducting a single experiment was as follows: The three Paramecia were sealed in the fluorite depression chamber and one of the three organisms (hereinafter designated organism A) was exposed to the fluorite rays. The depression chamber containing all three organisms was then placed in the warm stage and the organisms subjected to the increased temperature for the desired length of time, after which the depression slide was removed from the warm stage and cooled to room temperature. Organism B was then exposed to fluorite rays—the length of the exposure being the same as that received by organism A. Organism C, which was not radiated at all, served as a control for the effects of the heating, and as a test for the presence of ozone.

All three organisms were thus exposed to the same degree of heat for the same length of time. Organisms A and B received, in addition, an exposure to fluorite rays, and as far as we can judge, they received the same amount of radiation. The difference lies in the order in which the radiation and heat were received. The experiments show that this order is an important factor influencing both the rate and the amount of the resulting cytolytic changes.

As a matter of fact, this full program for the conduction of a single experiment was not always carried out, especially in the later experiments. It was more difficult to radiate one organism without exposing the others if three organisms were present in the depression chamber than if only two were present. Organism C was easily confused with organism B, since both appeared normal. As soon as we had satisfied ourselves that the temperature and other conditions to which the control Paramecia were subjected were not lethal, we omitted the heat control, as the organism receiving heat before the radiation was sufficient control for the effects of heat and for the influence of any toxic

substances which might be formed by the photo-chemical effects of the fluorite rays on the culture medium.

The length of exposure used in these experiments was thirty seconds. Eighty-five per cent of the organisms had ceased locomotion at the end of the exposure. With this exposure vesicle formation, instead of occurring within a few seconds after locomotion ceased, was delayed about one-half hour, while the average time required for complete cytolysis was over one hour. Only 17 per cent of the radiated organisms were cytolyzed at the end of fifty minutes.

According to Woodruff and Baitsell (6), the optimum temperature range for growth and reproduction lies between 24° and 28.5°C. We found a temperature of 37° to 38°C. for three minutes to be lethal. At this temperature there is a rapid formation of vesicles, followed by complete cytolysis.

The organisms used in these experiments were cultured at room temperature (about 22°C.). For the heat sensitization experiments they were exposed to temperatures of 27.5°, 30° or 35°C. for five minutes. In one set of experiments they were exposed to a temperature of 30°C. for fifteen minutes. These temperatures for the periods of time used are all sublethal to normal Paramecia.

We ran one series of fourteen experiments at 36.7°C. for three minutes, i.e., just below the lethal temperature. The results of these experiments were not different in kind from those of experiments run at lower temperatures, except that, as was to be expected, the heat effect was more pronounced.

The organisms were observed under the microscope during the entire experiment. The length of time after the exposure, at which definite changes in the organisms such as resumption of normal activity, vesicle formation, rupturing and internal disorganization took place, was recorded. Any one of these cytolytic changes might have been used as an "end point" to give our results a numerical expression. We have selected vesicle formation for the experiments tabulated below. Vesicle formation is a conspicuous change and except in the few cases of recovery mentioned above, it was always followed by complete cytolysis. The results of the experiments are shown in the tables 1 to 5.

The significance of the data tabulated above will be made more apparent if we tabulate the results obtained as a successive summation of the total number of organisms which have formed vesicles at the end of five-minute intervals after receiving the "A" or "B" treatment. In order to compare one series of experiments with another we will indicate the percentages of the number of organisms exposed, instead of the actual numbers (table 6).

TABLE 1

*Paramecia exposed to a temperature of 34.6°C. ± 1.0°C. for a period of 5 minutes*

| | | LENGTH OF LATENT PERIOD IN MINUTES | | | | | | | | | | | | | | NUMBER OF PARAMECIA NOT FORMING VESICLES | TOTAL NUMBER OF ORGANISMS EXPOSED |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 12 | 15 | 23 | 28 | | | |
| Number of Paramecia forming vesicles | A | 5 | 8 | 9 | 5 | 7 | 4 | 2 | 1 | | | 1 | 1 | 1 | | 6 | 50 |
| | B | 1 | 3 | 1 | 3 | 1 | | | 1 | 1 | 1 | | | | | 38 | 50 |

TABLE 2

*Paramecia exposed to a temperature of 30.5°C. ± .6°C. for a period of 5 minutes*

| | | LENGTH OF LATENT PERIOD IN MINUTES | | | | | | | | | | | | | | | | | | | | NUMBER OF PARAMECIA NOT FORMING VESICLES | TOTAL NUMBER OF ORGANISMS EXPOSED |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 | 3 | 4 | 5 | 6 | 8 | 10 | 11 | 12 | 13 | 15 | 16 | 17 | 19 | 22 | 40 | 41 | 51 | 60 | 80 | | |
| Number of Paramecia forming vesicles | A | 2 | 3 | 0 | 1 | 1 | 3 | 1 | 1 | 0 | | 2 | | 1 | 1 | 1 | 1 | | 1 | | 1 | 15 | 35 |
| | B | 1 | 0 | 1 | | | | | | 2 | 1 | | 1 | 1 | | | | 1 | | 1 | | 26 | 35 |

TABLE 3

*Paramecia exposed to a temperature of 27.5° ± .4°C. for a period of 5 minutes*

| | | LENGTH OF LATENT PERIOD IN MINUTES | | | | | | | | | | | | | | | | | | | | | | NUMBER OF PARAMECIA NOT FORMING VESICLES | TOTAL NUMBER OF ORGANISMS EXPOSED |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 4 | 5 | 7 | 8 | 9 | 10 | 12 | 13 | 14 | 17 | 22 | 23 | 41 | 42 | 50 | 60 | 73 | 122 | 135 | 150 | | |
| Number of Paramecia forming vesicles | A | 2 | 1 | | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | | | 1 | 1 | | | 1 | 1 | 1 | | 1 | 14 | 30 |
| | B | | | 1 | | | 1 | | 2 | | | | 1 | 2 | | | 1 | 1 | 1 | 1 | | 1 | | 18 | 30 |

TABLE 4

*Paramecia exposed to a temperature of 30.2 ± .7°C. for a period of 15 minutes*

| | | LENGTH OF LATENT PERIOD IN MINUTES | | | | | | | | | | | | | | NUMBER OF PARAMECIA NOT FORMING VESICLES | TOTAL NUMBER OF ORGANISMS EXPOSED |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 7 | 9 | 10 | 12 | 14 | 15 | 18 | 63 | 98 | | |
| Number of Paramecia forming vesicles | A | 2 | 1 | 3 | 1 | 1 | 1 | | | 1 | 1 | 1 | 1 | | 1 | 4 | 18 |
| | B | | | 1 | | | | 1 | 1 | | | | | 1 | | 6 | 10 |

TABLE 5

*Paramecia exposed to fluorite radiation alone*

| | LENGTH OF LATENT PERIOD IN MINUTES | | | | | | NUMBER OF PARAMECIA NOT FORMING VESICLES | TOTAL NUMBER OF ORGANISMS EXPOSED |
|---|---|---|---|---|---|---|---|---|
| | 1 | 4 | 5 | 10 | 15 | 90 | | |
| Number of Paramecia forming vesicles | 1 | 1 | 1 | 2 | 1 | 2 | 22 | 30 |

Table 6 is represented graphically in figures 1 and 2, in which the percentages are plotted as ordinates against the length of the latent period in minutes as abscissae.

TABLE 6

*Rate of vesicle formation*

A summation table showing the percentage of the total number of organisms in each series of experiments which had formed vesicles at end of 5-minute intervals

| LENGTH OF LATENT PERIOD IN MINUTES | A | A | A | A | B* | RADIATION WITHOUT HEAT |
|---|---|---|---|---|---|---|
| | 34.6° ± 1.0°C. FOR 5 MINUTES | 30.5° ± 0.6°C. FOR 5 MINUTES | 27.5° ± 0.4°C. FOR 5 MINUTES | 30.2° ± 0.7°C. FOR 15 MINUTES | 34.6°, 30.5°, 27.5°C. FOR 5 MINUTES | |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 5 | 68.0 | 17.4 | 13.3 | 44.0 | 9.0 | 10.0 |
| 10 | 82.0 | 31.9 | 23.3 | 49.5 | 13.7 | 16.6 |
| 15 | 84.0 | 40.6 | 33.3 | 66.0 | 17.2 | 20.0 |
| 20 | | 46.4 | | 71.5 | 21.2 | |
| 25 | 86.0 | 49.3 | 36.6 | | 23.4 | |
| 30 | 88.0 | | | | | |
| 40 | | 52.2 | | | | |
| 45 | | | 40.0 | | 25.4 | |
| 50 | | | | | 26.5 | |
| 55 | | 55.1 | | | 27.5 | |
| 60 | | | 43.3 | | 29.6 | |
| 75 | | | 46.6 | | 30.7 | |
| 80 | | 58.0 | | | | |
| 90 | | | | | | 26.6 |
| 100 | | | | 77.0 | | |
| 125 | | | 50.0 | | | |
| 135 | | | | | 31.9 | |
| 150 | | | 53.3 | | | |
| Final per cent forming vesicles..... | 88.0 | 58.0 | 53.3 | 77.0 | 31.9 | 26.6 |
| Percentage of total number of organisms not forming vesicles during observation..... | 12.0 | 42.0 | 46.7 | 23.0 | 68.1 | 73.4 |
| Total..... | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

* The length of the latent period for vesicle formation in the organisms exposed to fluorite rays after the exposure to heat (organisms designated B in the tables above) appears to be independent of the temperatures used. We have, therefore, given the averages of the summation of all of the three temperatures used.

As will be seen from figure 1, the organisms which received the exposure to heat after the exposure to radiation showed a greater degree of injury than those receiving the exposure to heat before the radiation. Organisms which have been exposed to fluorite radiation are sensitized to heat.

It will also be seen that curves *B* and *C* are similar. The two curves so nearly coincide that it is quite evident that it is the exposure to fluorite radiation rather than the exposure to heat which is the essential condition of vesicle formation in the B series. In fact, we are unable to observe that the exposure to temperatures above normal before the exposure to radiation has any effect either on the degree of, or on the rate of the resulting photo-cytolysis. The increase in the effect is, therefore, not due merely to the added injury of radiation and heat. The sequence in which these injuries are received is the determining factor. That we are dealing with a case of sensitization to heat is further shown by the results of the experiments in which the organisms were exposed to a temperature of only 27.5°C,—a temperature which is within the range of the optimum temperature for growth and reproduction of normal Paramecia.

The duration of the exposure to heat remaining the same, the degree of sensitization varies directly with the temperature to which the organisms are exposed (fig. 1). The temperature remaining the same, the degree of sensitization varies directly with the duration of the exposure (fig. 2).

According to Woodruff and Baitsell (7), at a temperature of 32°C. normal Paramecia die in about two days. The high percentage of organisms forming vesicles after a comparatively brief exposure to heat, as shown by the *A* curves, is additional evidence that an exposure to fluorite rays renders the protoplasm more sensitive to heat.

It will be seen therefore that the results of these experiments are fundamentally different from those of Rohdenburg and Prime (8), who found that the combined effects of a certain exposure to heat alone, which was not lethal, and of x-rays alone, which was not lethal, were lethal when the two were combined, the sequence of the exposures being apparently immaterial. As has been pointed out by Greenough (9), these experiments of Rohdenburg and Prime are not quite comparable with those of the experiments described in this paper because in these experiments the sequence of the exposures is the determining factor.

The mechanism through which the fluorite rays bring about this increased sensitivity of Paramecium protoplasm to heat is not known. The sensitization of egg albumen to heat by an exposure to quartz ultra-violet rays (10) is clearly suggested.

FIGURE 1.

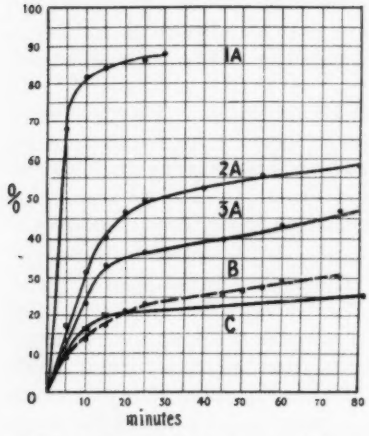



FIGURE 2.

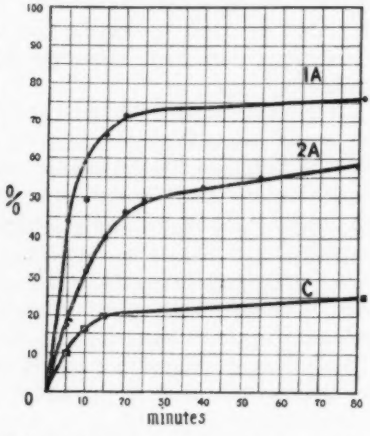



Fig. 1. A graphical representation of the influence of temperature on the length of the latent period.

The percentage of the total number of organisms in each series of experiments which have formed vesicles is measured on the ordinate axis. The length of the latent period is measured on the abscissa axis.

Curve *1A*. Organisms heated to a temperature of 34.6°C. ± 1.0°C., for 5 minutes after the exposure to fluorite rays.

Curve *2A*. Organisms heated to a temperature of 30.5°C. ± 0.6°C., for 5 minutes after the exposure to fluorite rays.

Curve *3A*. Organisms heated to a temperature of 27.5°C. ± 0.4°C., for 5 minutes after the exposure to fluorite rays.

Curve *B*. Average of all organisms heated for 5 minutes before the exposure to fluorite rays.

Curve *C*. Organisms exposed to fluorite rays alone, not heated.

Fig. 2. A graphical representation of the influence of temperature on the length of the latent period.

The percentage of the total number of organisms in each series of experiments which have formed vesicles is measured on the ordinate axis. The length of the latent period is measured on the abscissa axis.

Curve *1A*. Organisms heated to a temperature of 30.2°C. ± 0.7°C., for 15 minutes after the exposure to fluorite rays.

Curve *2A*. Organisms heated to a temperature of 30.5°C., ± 0.6°C., for 5 minutes after the exposure to fluorite rays.

Curve *C*. Organisms exposed to fluorite rays alone, not heated.

In these tables: The duration of exposure to the fluorite rays was thirty seconds.

The time elapsing between the end of the exposure to fluorite radiation and the appearance of vesicles is called the "latent period."

Organisms exposed to fluorite radiation before the exposure to heat are designated by the letter A.

Organisms exposed to fluorite radiation after the exposure to heat are designated by the letter B.

The existence of a latent period means that after the exposure to fluorite rays, certain changes, probably metabolic, must occur in the protoplasm before cytolysis is initiated. If no changes occurred the latent period would last indefinitely. The internal energy of certain atoms or atomic groups of protoplasm which have absorbed fluorite radiation (perhaps in units of quanta, as Einstein has suggested) is increased so that they are changed from a chemically passive to a chemically active condition. According to Baly's theory of isorropesis, as a result of the absorption of radiation their "valency force fields are opened up" and atomic rearrangements must take place before chemical equilibrium is restored. The reaction velocities of the various chemical changes occurring in the protoplasm are not affected alike and the nice balance of chemical change is upset. As a result of these photo-chemical changes some of the metabolic processes do not proceed at the usual rate and the normal physiological correlation does not occur. Evidence of this lack of correlation has already been seen in the failure of the locomotor mechanism.

It is not difficult to understand how an increase in temperature might, by increasing the rate of some of the metabolic changes, augment this misrelation and thus hasten that physiological dissociation which is called death.

This course of events may not be specific for fluorite radiation; any chemical or physical agent might act in an analogous manner. However, we have not observed heat sensitization following any other form of injury to Paramecium and we do not believe the phenomenon to be common else it would be referred to in the more or less extensive literature on this organism. The uniqueness of the biological effects of radiation lies in the fact that the rays form within the cell chemical combinations which are foreign to the normal protoplasm. Like the four dimensional beings described in A Square's "Flat Land," we are able to introduce our toxic material (photo-products) into the interior of the cell without passing it through the limiting membrane.

It must be evident that the interaction of these photo-products with the normal protoplasm depends not only upon the nature of the formed substance but quite as much upon the structure and nature of the surrounding protoplasm. The failure of Rohdenburg and Prime to observe heat sensitization may have been due to the comparatively inactive condition of the tumor tissues at the time they were exposed to the heat. Clinical studies might or might not give similar results.

These heat sensitization experiments serve to emphasize the fact that we observe disturbances of function only where we know how to look for them. The radiated organism which has not been exposed to an increased temperature may appear quite as normal as if the rays

had no effect whatsoever. It is only when the initial photo-chemical change, "latent image," has been "developed" by the metabolic changes occurring at the higher temperature that the effects of the exposure are to be observed. An apparent specificity of the action of rays may be nothing more than a reflection of our experimental methods. The investigator himself is hypersensitive to those physiological disturbances which he has learned to observe.

We cannot lay down fundamental principles concerning the specific nature of the action of rays. Whether or not the phenomenon of heat sensitization by radiation is general, can be established only by further investigation.

## BIBLIOGRAPHY

(1) FORBES AND DALAND: This Journal, 1923, lxvi, 50.
(2) BOVIE AND KLEIN: Journ. Gen. Physiol., 1919, i, 331.
(3) WOODRUFF AND BAITSELL: Journ. Exper. Zoöl., 1911, xi.
WOODRUFF: Biol. Bull., 1909, xvii.
(4) BOVIE: Bot. Gazette, 1916, lxi, 1.
HUGHES AND BOVIE: Journ. Med. Research, 1918, xxxix, 233.
(5) LYMAN: Astroph. Journ., 1908, xxvii.
(6) WOODRUFF AND BAITSELL: This Journal, 1911–12, xxix, 147.
(7) WOODRUFF AND BAITSELL: Ibid.
(8) ROHDENBURG AND PRIME: Journ. Cancer Research, 1921, vi, 101.
(9) GREENOUGH: Ibid.
(10) BOVIE: Science, N. S., 1913, xxxvii, 373.

# STUDIES ON THE PHYSIOLOGY OF SLEEP

## I. The Effects of Prolonged Sleeplessness on Man

NATHANIEL KLEITMAN

*From the Hall Physiological Laboratory of the University of Chicago*

Received for publication May 28, 1923

Most investigators of the physiology of sleep, in reporting their findings, remind their readers, by way of apology, of the tremendous importance of the subject for the advancement of our knowledge of physiology as a science, as well as for the rational treatment of insomnia. They also like to record the fact that the average individual spends more than a third of his life in sleep, has been doing this from time immemorial, and raise the question whether eight hours or more of sleep a day really constitutes the minimum penalty for keeping awake the rest of the time. It seems reasonable to suspect that as in the case of protein consumption, there is a large "factor of safety" in the amount of sleep we are getting, and that it could be considerably reduced without impairment of health or loss of efficiency. This and other questions related to industrial physiology, especially physiology of fatigue, can be answered only by a thorough systematic study of the subject. But curiously enough, there are scarcely a dozen investigators engaged at any one time in the study of this great physiological mystery, and most of these workers are psychologists. It seems that because animals are not very well adapted for this work and the human beings that are available cannot be dissected, physiologists have allowed the psychologists to tackle this problem as best they could. Present day textbooks of physiology contain but a few short references to the subject, usually antiquated, and some texts (Starling's, for example) ignore the subject entirely. Luciani's four-volume work is the only textbook that treats the subject adequately, but unfortunately it is not in general use as a manual. Thus most medical students first encounter sleep in their study of pharmacology, and there they learn several easy ways of putting a person "to sleep." In this manner the "practical" aspect of the problem for them at least is solved. If we recall that textbook writers generally do not peruse psychological literature in bringing out their new editions, it will be readily understood why the work of many able investigators has not been brought to the attention of our students.

In this paper references will be made to both physiological and psychological literature. One of the best books ever written on the subject of sleep is Piéron's *Le Problème Physiologique du Sommeil* (1), which besides embodying the results of original research on sleep from the histological, biochemical and physiological standpoints (this by a psychologist!) contains a valuable and exhaustive bibliography brought up to 1912. To save space references will be given only to those papers which appeared after 1912, or have not been included in Piéron's bibliography; others referred to as (P) will be found in Piéron's bibliography.

Sleep should be studied not only in man, but in animals as well. Little work has been done on the comparative physiology of sleep, and at present we have no good criterion for determining whether an animal is asleep or not. Thus some people endow even plants with the ability to sleep, while others restrict sleep to animals with a nervous system, "no matter how rudimentary" (8). In these studies experiments were made on men and dogs, and this, the first paper of the series, contains a report of the results obtained on the human subject.

Methods. The method of experimental insomnia appeared to be best suited as a beginning, for two reasons. In the first place, it should enable us to study the behavior and reactions of a subject deprived of sleep for a number of days, the concomitants of prolonged wakefulness and, if possible, to see whether all the changes that the various theories of sleep postulate actually occur; and in the second place, it should permit us to observe the concomitants of deep dreamless sleep, such as we could expect the subject to lapse into at the end of the period of experimental insomnia. The first to use this method on man were Patrick and Gilbert (P) who in 1896 kept three persons awake for a period of 90 hours. They performed a number of psychological and physiological tests during the experiment and on the day following the completion of the experiment, but had no normal control with which to compare their results. The test was made only once, and the individual findings will be referred to in various places in the course of this and following papers. In 1916 Smith (3) performed a number of psychological tests upon herself, studying primarily the effects of fatigue. On some occasions she studied the effects of the loss of one night's sleep. More recently Robinson and Herrmann (4) reported results obtained on three subjects who had been subjected to a period of experimental insomnia lasting 65 hours. They tested the subjects' muscular strength (Smedley's hand dynamometer), steadiness, aiming, ability to name letters and to do mental arithmetic. In this case the period of sleeplessness was preceded and followed by normal periods of observation of some days' duration (11 to 26 before and 4 to 5 after the test). They likewise performed the insomnia experiment only once.

In this study we employed six subjects, all young adults, male students at the University of Chicago. Each of these went through one or more sleepless periods. The duration of the experimental insomnia varied in different tests from 40 to 115 hours. Thus as regards the number of subjects used, the number of periods of insomnia and the maximum duration of sleeplessness, this method has been considerably extended by our experiments. Most of the work that involved long periods of normal control testing was done on the writer, who went through more than a dozen sleepless periods himself. The other subjects were used to check the results obtained on him.

The original intention was to keep conditions as uniform as possible and to have only one variable—absence of sleep. The subject was supposed to undress and go to bed at the usual hour, to relax his muscles, and keep awake throughout the night. This was found impossible of accomplishment, because the subject always fell asleep some time during the night. Attempts to keep awake by sitting in a reclining chair were likewise unsuccessful. The only method that was found to work consisted in having the subject keep moving throughout the night, with short periods of rest sitting down.. Even in that case he would fall asleep shortly after he sat down, and he therefore had to be watched continually throughout the period of insomnia, especially at night-time. On some occasions even this method was only partially successful, because the subject would fall asleep as soon as he was awakened, and unless the observer made him walk it was impossible to keep him awake. For these reasons the experiments were not *strictly* insomnia tests, but insomnia plus a certain amount of muscular activity during the night, activity that a person would not engage in if asleep. The results obtained and reported below may be partly at least due to this increased muscular work. But from the standpoint of intoxication theories of sleep this could not be considered a drawback. On the contrary, the intoxication, if present, should only be accentuated by the products of muscular activity. On the other hand, the perpetual falling asleep during certain hours of the night might be looked upon as short almost momentary "naps," and it might be argued that they would have some restorative power, and would enable the subject to go without sleep longer than he could otherwise. The method then is not perfect, but it is the best we could devise at this time.

For the determinations of the pulse, temperature, respiratory rate and blood pressure, the subject was allowed to lie completely relaxed for at least 15 minutes, and in measuring basal metabolism, for at least 30 minutes. Only in one series of tests were the measurements taken with the subject in the sitting position, and this for purposes of comparison. All these determinations were made daily at 7 a.m. The

pulse was counted by ordinary palpation of the radial artery, the count being made after the subject's hand had been held for over one minute. Respiratory rate was determined by observing the movements of the pointer on the cylinder of the spirometer at a time unknown to the subject. Temperature was measured by a one-minute certified clinical thermometer, which was kept under the tongue for at least five minutes. For blood pressure we used the Tycos sphygmomanometer, making all determinations in duplicate. Basal metabolism was measured by means of the Benedict portable respiration calorimeter (5), using as a basis oxygen consumption only. Analysis of the blood was usually made late in the afternoon. For the red and white blood count we used the Neubauer counting chamber. Hemoglobin was determined by means of Sahli's hemoglobinometer, using Dare's hemoglobinometer as a check. For blood sugar determinations the Folin-Wu method was used, and the alkaline reserve of the blood or the plasma was determined by the Van Slyke method. The percentage of corpuscles was determined by the hematocrit method.

In our urine work we partitioned the 24-hour sample into two parts. What we call the day urine is a 16-hour sample from 6 a.m. to 10 p.m.; the night urine, an 8-hour sample from 10 p.m. to 6 a.m., the period devoted to sleep. All previous workers divided the 24-hour urine into two 12-hour samples, their "night urine" including not only the urine excreted during the eight hours of sleep, but also that of four hours of wakefulness. Thus the data we present show the activity of the kidney during wakefulness and during sleep, normally and during the hours usually devoted to sleep in our periods of experimental insomnia. We also studied the activity of the kidney during periods of "reversed routine," that is, sleep during the day and wakefulness at night. Campbell and Webster (6) have recently reported similar experiments on the secretory activity of the kidney on a reversed routine, but they allowed their subjects to eat at night, thus introducing new factors. In our experiments in which two subjects were used for many weeks, the meal hours were fixed at 8 a.m., 12 m. and 9:30 p.m., so that the subject on "reversed routine," sleeping from 1 p.m. to 9 p.m. did not have to change his meal hours. No attempt was made to keep the diet absolutely uniform, and these are to be regarded not as metabolism experiments, but as a test of the secretory rhythm of the kidney and its possible persistence or change during changed conditions. In the urine analysis, for total nitrogen the modified Kjeldahl method was employed, for creatinine the Folin method, using pure creatinine solution as a standard; chlorides were determined by the ordinary Vollhard method; phosphates by the uranium acetate method; and total acidity by the Folin titration method.

In the tests made during the sleep following insomnia the heart rate, respiratory rate, temperature and blood pressure were determined as above. The pupillary reflex and the position of the eyes were examined by gently spreading the eyelids with the fingers, and flashing a light from a pocket flashlight. The skin of the face was stimulated by touching it with sharp pieces of paper, and the reflex movements of the arms and hands on both sides were observed. The plantar reflex was determined by passing a pencil along the inside and outside aspects of the soles, the foot being illuminated by a flashlight. The subjects very rarely woke during these tests, and their general reflex behavior was observed during the several determinations.

RESULTS: *Subjective observations during insomnia.* While there were differences in the subjective observations of the subjects in the course of the experimental insomnia, they were so slight that a common description for them all will suffice. During the first night the subject did not feel very tired or sleepy. He could study or read or do laboratory work, but felt an attack of drowsiness between 3 and 5 o'clock in the morning. There were some itching or burning sensations in the eyelids during the night. In the morning the subject felt as usual, except for a slight indisposition which always appeared when the subject sat down to rest. As long as he kept moving, and especially if he engaged in laboratory work, he did not feel any urgent desire to sleep. On the second night he felt sleepier much earlier, or else felt a peculiar "buzzing in the head," or else a sensation of emptiness, not only in the head but in the entire body. Similar sensations were described by the subjects of Patrick and Gilbert and of Robinson and Herrmann. It was more difficult to keep awake during the second night, and the dryness of the eyes often became very unpleasant. It was found impossible to read or to study, because sitting would cause the subject to fall asleep. However, even during the second night work requiring manipulations and movement from place to place could be carried on with great ease and had the effect of banishing the desire for sleep. On the day following the longing for sleep subsided again, and the person could perform routine work as usual. He had great difficulty, however, in keeping awake at lectures. Even if he did not fall asleep while listening to the monotone of the lecturer, he had difficulties in taking notes. After a few words had been written in the correct fashion, his hand would begin to slip in executing the delicate movements of writing, would slide across the paper, and instead of words there was unintelligible scribbling. A new effort would make the hand write properly, but only for a short time. The lecture hour was a trying one for every one of our subjects. On the third night the desire for sleep was still more accentuated, but on the day following the subject again felt much better. On this day

taking lecture notes was entirely impossible, as the pencil would fall out of the subject's hands after he had been sitting for a short while. Laboratory work was possible, as on previous days. Attempts to count one's own pulse were almost always unsuccessful, because the subject would lose trend of the count after he had reached twenty or thereabouts, or else would become extremely drowsy. Only one subject (N. K.) kept awake for a fourth night, and he reported momentary dreams during the night, whether walking, standing or sitting. No hallucinations had been experienced by any of our subjects. The subjective feeling of drowsiness was not any worse after 115 than after 60 hours of wakefulness, and if the subject was engaged in an interesting conversation, he felt no desire for sleep at any time during the experiment. Likewise, a trip to an "all-night" café proved to a number of our subjects that interest in their surrounding had a powerful effect in dispelling all the subjective symptoms of drowsiness and depression. Lying down had the effect of inducing immediate sleep. This was observed on the subject whose basal metabolism was measured every morning during the period of insomnia, and who had to relax completely for this purpose. The observer had to keep a very close watch over him, and even so he managed to fall asleep while his companion determined his blood pressure (this may partially account for some of the low blood pressure values obtained). Usually on lying down for a minute or so the eyelids drooped without closing (relaxation of the levator palpebrae super.), and the subject saw double because of the divergence of the eyeballs. Shortly after that the eyes closed, unless the subject was disturbed. If the subject was aroused as he was falling asleep in the horizontal position, it was a matter of seconds before he was asleep again. We may sum up by stating that subjectively all the individuals tested felt sleepier during the night than during the day following; that drowsiness could be dispelled by any form of muscular activity and sleepiness accentuated by inactivity; that it was more difficult to stay awake while sitting than while standing and entirely impossible while lying; that at times the subject felt a greater need to sit down or lie down and rest than to sleep. None of the subjects employed experienced any loss or even diminution of appetite during the period of insomnia.

*Objective observations during insomnia.* In appearance the victim of experimental insomnia did not differ from his fellows. In fact no observer could tell a person who had not slept for several nights from a normal individual. This was true, however, only of the subject who was engaged in some kind of activity. When he sat down, one could see that he was extremely sleepy, even in the daytime. At night the observer had to watch the subject very closely. It was amusing to

note that some subjects used ruses to escape the watchful eye of the observer. After being roused many times they pretended to get up for a stroll in the corridors of the building, but would actually walk over to some corner, sit down and fall asleep almost immediately. On other occasions the subjects would become irritable and resent being aroused every few minutes. They would pretend that they were not falling asleep, would roundly deny the fact. However, in all cases a short walk would restore the subject's good disposition and banish his drowsiness. The mental power of the subject was normal so far as could be determined. We performed a few tests on letter naming and on mental arithmetic, but our results were negative, confirming Robinson and Herrmann (4). Only in the subject who was kept awake for more than four days could some deficiency in mental power be observed. This consisted in temporary semi-dreaming states. In one case, early in the morning of the fifth day of insomnia, the subject was looking up logarithms of some numbers as they were called off by the observer. After some time the latter noticed that the subject's movements became mechanical and began to watch him as he looked up the various logarithms. He found that the subject invariably located the logarithms and called them off correctly, but once in response to a number whose logarithm he had located, instead of calling off the latter, said: "It is because they are against the system." On being questioned the subject admitted that all the time he was looking up the logarithms he had been under the impression that he was having a heated argument with the observer on the subject of labor unions. It is interesting to note that one of Patrick and Gilbert's subjects went through a similar experience during a weight lifting experiment, when instead of saying "number one," said "trimmings," and afterwards admitted to have dreamt during the experiment.

Our quantitative results will be found summarized in three tables. Patrick and Gilbert found a progressive increase in weight of their subjects during insomnia. We could not confirm this. Blood sugar and whole blood and plasma $CO_2$ determinations were made on two subjects (N. K. and M. P.). No variations that did not fall within the diurnal curve could be detected. Likewise, erythrocyte and leucocyte counts, percentage of hemoglobin and percentage of corpuscles, studied in one subject during several sleepless periods, showed no deviation from the normal. The absence of any change in the alkaline reserve of the blood is significant in that it shows that no toxic acid product accumulated in the blood during sleeplessness. The values for the heart rate, respiratory rate, temperature, blood pressure and basal metabolic rate, all obtained on subject N. K., are given in table 1. The normal days include the period preceding and following sleeplessness

tests, and the figures constitute the average of all the daily observations made. Besides the several periods of sleeplessness there was one 5-day period of complete fasting (4 observations) which was introduced mainly as a check on the accuracy of the basal metabolism determinations. In series 1, with the subject in the horizontal position, the average heart rate at 7 a.m. for 33 days was 74 per minute. The average obtained during three periods of insomnia (7 observations) was 62. In series 2, with 14 normal days and one period of insomnia of 4 days, the values are 75 and 62. The heart rate during insomnia was 17 per cent

TABLE 1

*Showing the effect of insomnia and fasting on the average heart rate, respiratory rate, temperature, systolic and diastolic blood pressure and basal metabolism*

| NUMBER OF DAYS | CONDITION OF SUBJECT | HEART RATE | RESPIRATORY RATE | TEMPERATURE | BLOOD PRESSURE | | BASAL METABOLISM |
|---|---|---|---|---|---|---|---|
| | | | | | Systolic | Diastolic | |
| | | | Series 1 | | | | |
| | | | | °C. | | | *calories* |
| 33 | Normal | 74 | 14 | 36.15 | 110 | 72 | 1491 |
| 7 | Insomnia | 62 | 14 | 36.07 | 105 | 67.5 | 1500 |
| 4 | Fasting | 80 | 14 | 36.20 | 114 | 77 | 1643 |
| | | | Series 2 | | | | |
| 14 | Normal | 75 | 14 | 36.50 | 107 | 72.5 | 1527 |
| 4 | Insomnia | 62 | 12 | 36.43 | 101 | 67 | 1569 |
| | | | Series 3 | | | | |
| 15 | Normal | 85 | 15 | 36.83 | 110 | 83 | |
| 4 | Insomnia | 79 | 13 | 36.80 | 116 | 88 | |

Subject N. K.; observations made daily at 7 a.m. In series 1 and 2 subject was in the lying position, in series 3 subject was seated.

lower than normal, and individual counts were as low as 56 per minute. In series 3, comprising 14 normal days and one 4-day insomnia period, with the subject in the sitting position, the decrease in heart rate resulting from sleeplessness was 6 beats per minute, or 7 per cent. During the fasting period the heart rate increased about 8 per cent. Respiratory rate showed no change in series 1, and a decrease of two respirations per minute in series 2 and 3. The temperature showed no change either during sleeplessness or during fasting. An interesting observation as regards the temperature variation, when it was recorded every two or three hours in several subjects during a number of tests, was the notice-

able "damping" of the diurnal temperature wave. A good example of this is shown in figure 1, which respresents the temperature curve during a 115-hour period of insomnia. The reader will note that the difference between the temperatures taken at 1 p.m. and 1 a.m. following was 1.38 degrees on the first day, 1.12 on the second, 1.00 on the third, 0.85 on the fourth and 0.68 on the fifth. While not all our temperature curves are as nearly diagrammatic as the one plotted, they all show the same tendency for the temperature to be equalized, indicating that the diurnal temperature rhythm is dependent upon sleep for its persistence.

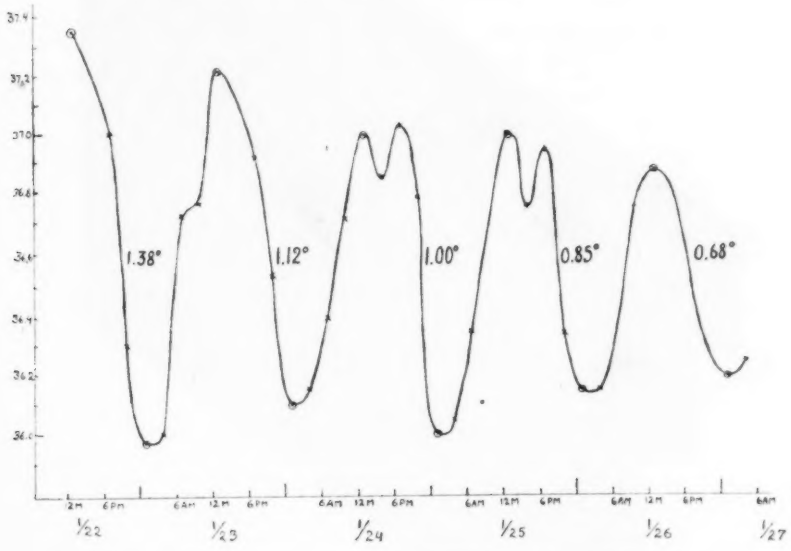



Fig. 1. Diurnal temperature curve during a 115-hour insomnia period. Circles on the curve indicate temperatures at 1 p.m. and 1 a.m. It will be noted that the difference between the temperatures taken at 1 p.m. and 1 a.m. becomes progressively smaller.

The blood pressure, both systolic and diastolic, was lower during the insomnia periods, when it was taken with the subject in the horizontal position; with the subject seated, it showed a rise. It also rose during fasting. The basal metabolic rate did not vary with lack of sleep. This may be only apparent, because the subject would continually fall asleep during the determination, and be continually awakened by the production of sharp metallic sounds. The momentary spells of sleep might have lowered the consumption of oxygen, while the awakenings involving as they do increased muscular tension, might have tended to

increase it. Comparative respiration curves on normal days and on sleepless days are shown in figure 2. On the whole there was a slight rise in basal metabolism during insomnia in both series 1 and 2. During

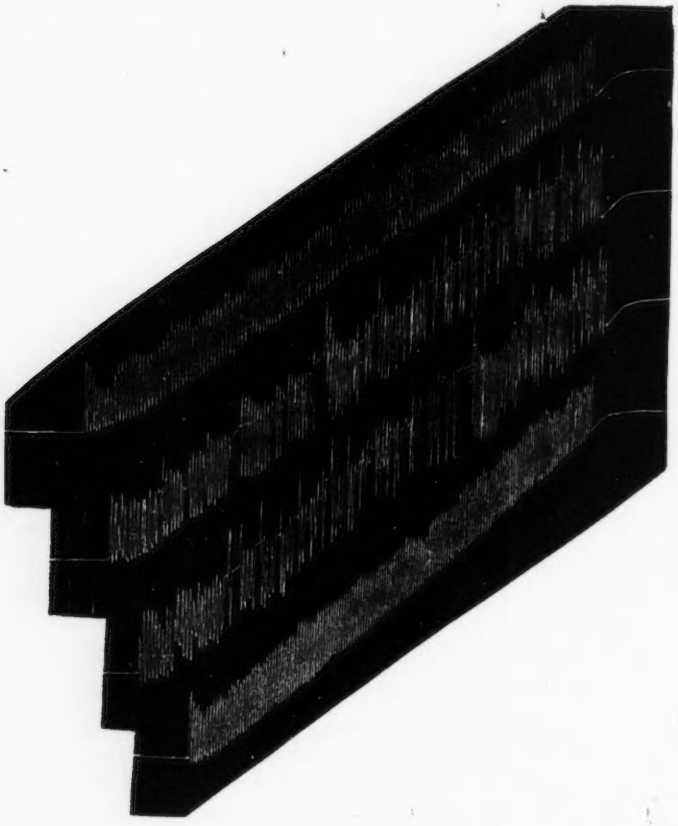


Fig. 2. Respiration tracings secured by means of the Benedict portable respiration calorimeter. Upper tracing taken on a day preceding the period of insomnia. The middle two tracings were taken on the third and fourth days of insomnia. The lowermost tracing was taken on the morning following the termination of the insomnia test. Reduced twice.

fasting the basal metabolic rate was markedly increased, which is in agreement with the results obtained by Kunde (7) under similar conditions.

*Observations during sleep following insomnia.* The subject of the insomnia experiment, at the end of the period, usually retired at 10 or 11 o'clock in the evening. The observer visited his bed-chamber at about 1 a.m. and again at 4 a.m. It was the invariable impression of the observers (as judged from general reflex irritability) that their subjects slept more deeply on the second visit than on the first. The values of the heart rate, respiratory rate, systolic and diastolic blood pressure and temperature taken during the first sleep following the period of insomnia in one subject are given in table 2. They show that

TABLE 2

*Observations on the heart rate, respiratory rate, temperature and blood pressure during sleep following period of insomnia*

| DATE | HEART RATE | RESPIRATORY RATE | TEMPERATURE | BLOOD PRESSURE | | DURATION OF INSOMNIA |
|---|---|---|---|---|---|---|
| | | | | Systolic | Diastolic | |
| First call—1 a.m. | | | | | | |
| | | | °C. | | | *hours* |
| 12- 3-22 | 71 | 12 | 35.60 | 97 | 62 | 64 |
| 12-13-22 | 63 | 12 | 35.80 | 98 | 66 | 90 |
| 1-27-23 | 68 | 12 | 36.20 | 94 | 62 | 115 |
| 2-13-23 | 66 | 12 | 36.10 | 103 | 76 | 65 |
| 2-24-23 | 75 | 14 | 36.00 | 98 | 76 | 115 |
| Average..... | 69 | 12.5 | 35.95 | 98 | 68 | |
| Second call—4 a.m. | | | | | | |
| 12- 3-22 | 60 | 11 | 35.70 | 96 | 64 | |
| 12-13-22 | 62 | 14 | 35.90 | 96 | 64 | |
| 1-27-23 | 63 | 12 | 36.25 | 95 | 65 | |
| 2-13-23 | 66 | 12 | 36.10 | 103 | 76 | |
| 2-24-23 | 72 | 12 | 36.10 | 101 | 74 | |
| Average..... | 64.5 | 12 | 36.00 | 98 | 68.5 | |

the respiratory rate was about the same at both hours, but that the heart rate was lower at 4 o'clock. In other subjects the respiratory and heart rates varied, sometimes being lower on the second observation, sometimes higher. The temperature did not vary from 1 to 4 a.m. The blood pressure was low, but not much lower than the pressure shown in the morning during the insomnia periods. The general behavior of the subject varied with the amount of sleep lost and with the usual depth of the person's sleep. Thus the sleep of one subject (N. F.) was normally much deeper than that of other subjects after two or three sleepless nights. But as a rule the sleep was deeper the greater the loss

of sleep during the preceding period of insomnia. The unavoidable sounds made by the observer rarely aroused the sleeper, but occasionally seemed to disturb him. The eyes were always found rolled up and out, but sometimes returned to their normal position wher the eyelids were spread apart. The pupils were frequently but not always found markedly constricted, but in one subject (S. L.) they were widely dilated. Every one of our subjects showed a positive pupillary reflex, but the response was very slow, it sometimes taking several seconds for the pupil to contract. There was no resistance to the insertion of the thermometer into the subject's mouth. The sleeper never closed his mouth tight enough to break the thermometer. The application of the sphygmomanometer cuff in some cases aroused the subject, but he always fell asleep before the reading was taken, as was evidenced by resumed snoring. Brushing the cheek with a rolled piece of tissue paper from the ear to the corner of the mouth often elicited a facial grimace and a movement of the hand, intended to scratch the stimulated spot. There was no strict homolaterality about this response. As a rule, the right hand would respond when the right cheek was stimulated, but if this hand was under cover and the left hand exposed, the latter would respond instead. In a few cases both a direct and a crossed reflex were elicited by this method. Plantar stimulation *invariably* elicited a positive Babinski in every one of our subjects during the sleep following insomnia, and in the deep sleeper referred to above (N. F.) during an ordinary night's sleep. Not only was there an extension but also a spreading of all the toes. This reflex could be obtained any number of times, provided a suitable interval (15 to 20 seconds) was allowed between successive stimulations. If the sole was scratched at shorter intervals, several extensor responses were followed by an extension-and-flexion, later by flexion of the toes, and either an attempt to rub the sole with the other foot or a flexion of the leg upon the thigh. On several occasions a crossed as well as direct Babinski was observed. Stroking the surface of the posterior tibial region from the tendo Achilles to the fossa of the knee usually resulted in a prompt flexion of the knee. All these tests were made without waking the sleeper, and if they did not follow each other too closely, without disturbing him. The duration of sleep following a period of insomnia was not much greater than normal.

*Analysis of urine.* In our urine work we first studied the excretion of total nitrogen in one subject (N. K.). The period covered 45 days of normal routine which preceded and followed two sleepless periods of 65 hours each, and one fasting period of 5 days. The normal average excretion of nitrogen was 14.477 grams in 24 hours, 9.911 grams for the day (16 hours) and 5.066 for the night (8 hours), or 0.619 gram and

0.633 gram per hour respectively. During the insomnia period the average was 15.323 grams in 24 hours, 10.538 grams for the day and 4.785 grams for the night, or 0.659 gram and 0.598 gram per hour. There was then a slightly greater excretion of nitrogen in the daytime during insomnia, but the difference is not great enough to make it significant. During the fasting period 11.805 grams of nitrogen were excreted in 24 hours, 7.730 grams for the day and 4.075 for the night (0.483 gram and 0.509 gram per hour respectively).

In a second series of experiments we used two subjects, N. K. and M. P. The results obtained are summarized in table 3. Both subjects had supposedly normal kidneys, and their urines showed neither albumen nor sugar. But when they were tested by means of the phenolsulphonephthalein excretion method, M.P.'s kidney function was found to be perfectly normal, whereas N.K.'s kidneys seemed somewhat below par. We are presenting the results obtained in both subjects, but as N.K. appears to be slightly nephropathic, more weight will be given to the figures obtained on M.P. From the table it will be seen that N.K. secretes more urine at night than in the daytime. That this increased secretion is dependent upon sleep can be seen from the fact that on a reversed routine he secreted more in the daytime than at night. M.P. secretes much more urine during the waking hours than while asleep, and the rate of the hourly secretion during the day was decreased on a reversed routine. During insomnia N.K. secreted less urine at night, further indicating that the increased night secretion under normal conditions was due to sleep. From these results it will be seen that the excess night secretion of urine by the nephritic or nephropathic kidney is connected not with the time of day, but with the condition of sleep. The phosphate excretion per 24 hours was almost twice as great in N.K. as in M.P. In either case, however, there was a slight diminution of phosphates on a reversed routine, and a slight increase during insomnia. N.K. excreted slightly less P at night than in the daytime, but this difference is greatly magnified (from 3.5 to 13.5 mgm. per hour) on a reversed routine. M.P. normally excretes much more P during the night, and this condition is strikingly changed on a reversed routine. The results obtained on both subjects show that a certain amount of the phosphate excreted bears a *direct* relation to sleep. The excretion of phosphates during the sleepless nights is less than normal in both subjects, confirming this conclusion. The same results were obtained by Campbell and Webster (6) on their 3- and 4-day periods of reversed routine, but, as stated above, in our experiments sleep was the only variable. Practically the same relations hold true for total acidity as for phosphates, confirming the observation of Campbell and Webster on the parallelism between the excretion of

TABLE 3

*The secretory activity of the kidney on a normal routine, "reversed" routine, and during experimental insomnia*

| SUBJECT | TYPE OF TEST | NUMBER OF DAYS | AVERAGE FOR 24 HOURS | AVERAGE FOR "DAY" | | AVERAGE FOR "NIGHT" | |
|---|---|---|---|---|---|---|---|
| | | | | 16 hours | 1 hour | 8 hours | 1 hour |
| **1. Volume of urine in cubic centimeters** | | | | | | | |
| N. K. | Normal | 45 | 1063 | 680 | 43 | 383 | 48 |
| | Reversed | 4 | 1054 | 749 | 47 | 305 | 38 |
| | Insomnia | 8 | 1009 | 700 | 44 | 309 | 39 |
| M. P. | Normal | 35 | 1498 | 1173 | 73 | 325 | 41 |
| | Reversed | 12 | 1422 | 1090 | 68 | 332 | 42 |
| **2. Phosphates, in milligrams of P** | | | | | | | |
| N. K. | Normal | 45 | 1063 | 728 | 45.5 | 335 | 42 |
| | Reversed | 4 | 1027 | 808 | 50.5 | 219 | 27 |
| | Insomnia | 8 | 1121 | 821 | 51 | 300 | 37.5 |
| M. P. | Normal | 35 | 581 | 339 | 21 | 242 | 30 |
| | Reversed | 12 | 528 | 433 | 27 | 95 | 12 |
| | Insomnia | 2 | 647 | 418 | 26 | 229 | 29 |
| **3. Total acidity in cubic centimeters of N/10 acid** | | | | | | | |
| N. K. | Normal | 45 | 546 | 366 | 23 | 180 | 23 |
| | Reversed | 4 | 482 | 354 | 22 | 128 | 16 |
| | Insomnia | 8 | 530 | 360 | 23 | 170 | 21 |
| M. P. | Normal | 35 | 269 | 165 | 10 | 104 | 13 |
| | Reversed | 12 | 232 | 168 | 11 | 64 | 8 |
| | Insomnia | 2 | 217 | 104 | 7 | 113 | 14 |
| **4. Chlorides, in grams of Cl** | | | | | | | |
| N. K. | Normal | 45 | 6.01 | 4.47 | 0.280 | 1.54 | 0.193 |
| | Reversed | 4 | 5.40 | 3.54 | 0.221 | 1.86 | 0.233 |
| | Insomnia | 8 | 4.92 | 3.55 | 0.222 | 1.37 | 0.171 |
| M. P. | Normal | 35 | 6.57 | 5.07 | 0.317 | 1.50 | 0.188 |
| | Reversed | 12 | 4.65 | 3.19 | 0.200 | 1.46 | 0.183 |
| | Insomnia | 2 | 6.58 | 5.59 | 0.350 | 0.99 | 0.124 |
| **5. Creatinine, in milligrams** | | | | | | | |
| N. K. | Normal | 45 | 1988 | 1334 | 82 | 646 | 80 |
| | Insomnia | 8 | 2064 | 1438 | 90 | 626 | 78 |
| M. P. | Normal | 35 | 1862 | 1276 | 80 | 586 | 74 |
| | Reversed | 12 | 1934 | 1378 | 86 | 556 | 70 |

phosphates and the total acidity of the urine. Chlorides were excreted in greater quantities in the daytime than at night in both subjects tested. This rhythm persisted during insomnia, but there was a tendency toward reversal on the changed routine. This could not be due to a change in the ingestion of chlorides, as all meals were taken at fixed hours whether the subject slept at night or during the day. The figures for creatinine excretion are significant in that they show that more creatinine was excreted per hour in the daytime than at night, on the normal routine, reversed routine and during insomnia. It is the only substance of those we studied in reference to which the kidney shows a more or less fixed diurnal rhythm.

*Effects of muscular relaxation.* A number of tests were made on the effects of complete muscular relaxation. The subjects were asked to lie down and to relax their muscles. They were blindfolded and their ears were plugged with cotton. As a rule, after 30 minutes in this condition, the subject was found asleep. The observer did not return before 30 minutes so as not to unduly disturb the subject who could still hear somewhat with his ears plugged. Sidis (P) and Coriat (8) have reported similar experiments on children and adults respectively, and our results confirm theirs, although we are inclined to agree with Coriat's rather than Sidis' interpretation. But this will be taken up in the next section.

Discussion and theory. What is sleep? We must first agree as to the facts, or the so-called concomitants of sleep, and only then attempt to interpret them. The definitions given by most workers agree with each other pretty closely, and the one proposed by Piéron (1) seems to be the best. According to this investigator, sleep is a suspension of the sensori-motor activities that bring the living being into relation with its environment. It is characterized by *a*, marked diminution of muscular tonus in general (with the exception of sphincters and a few other muscles) and the loss of the power of equilibrium; *b*, almost complete abolition of "spontaneous" activity; *c*, raising of the threshold of reflex irritability and general sensibility; and most important, *d*, complete absence of critical reactivity, which normally involves analysis of sensations. All other rest states seen in animals, whether diurnal or seasonal, could not be looked upon as sleep, but, together with hypnosis, coma, etc., as sleep-like conditions.

Piéron divides the numerous theories of sleep into "partial" and "complete" theories. By a partial theory he means one that attempts to explain the mechanism of going to sleep, or of the onset of sleep, without attempting to explain the biological necessity of sleep in higher animals; complete theories "explain" not only how we fall asleep, but also why we sleep at all. This division is not very appropriate, because

a theory that explains quite adequately why we sleep, may not have a reasonable explanation of the mechanism of the onset of sleep. Besides, since sleep involves loss of consciousness and of critical reactivity to our surroundings, it would seem that until we know everything concerning the *physiology* of consciousness as contrasted with its psychology, i.e., the chemical and nervous mechanism we shall not know what the loss of consciousness is. For this reason those parts of our present so-called "complete" theories which explain the cause of sleep are not susceptible of experimental confirmation or refutation, and the main concern of an author of such a theory is that it should be in harmony with as many known facts about sleep as possible, and not in glaring contradiction with the rest. On the other hand, "partial" theories, or those parts of the complete theories dealing only with the mechanism of the onset of sleep, can be easily challenged experimentally. These theories are of immediate practical importance in the solution of that type of insomnia which is characterized by the patient's inability to fall asleep. A good "partial" theory should also explain the mechanism of awakening.

Of the theories of the mechanism of the onset of sleep Piéron discusses and criticizes half a dozen groups, and we shall merely touch upon the vasomotor theories of which Howell (P) is a proponent in this country. These theories assume that at the end of the day's activities the vasomotor center becomes fatigued, and this periodic loss of tone of the center is responsible for the inadequate circulation through the brain. This theory is partly based on Mosso's statement (P) that the brain is anemic during sleep. Shepard has recently brought forward evidence to show that during sleep there is a plethora of the brain (9). In our experiments we could not detect any loss of tone of the vasomotor center even after 115 hours of wakefulness, or 7 times the normal 16-hour period. The low blood pressure obtained during the insomnia periods was partly due to a slowed heart, and both the slowed heart and low blood pressure were due to muscular relaxation in the lying position, and were absent when the subject was seated. The facts that it was impossible to keep awake while lying down, in which position the demands on the vasomotor center are small, and that it was easy to keep awake while very active are against this theory. As Shepard says, the vasomotor change, be it anemia or plethora, may just as well be considered a consequence as a cause of sleep. We dwelt on this theory at some length because it is so "popular."

Another outstanding theory is that of auto-intoxication with the products of wakefulness. Piéron reports a number of remarkable experiments on dogs, which we will discuss in a subsequent paper. He discovered a substance, "hypnotoxin," which can be found in the

cerebro-spinal fluid of dogs kept awake for a number of days or weeks and which, when injected into the fourth ventricle of a normal dog will produce somnolence and later sleep. Piéron does not claim, as text-books maintain, that at the end of a normal day enough "hypnotoxin" accumulates to cause the animal to fall asleep. At best, he thinks, this substance developed during the intensive activity of the nervous centers which preside over the extremely complex sensorimotor functions brings on sleep by reflex inhibition of these centers and *not* by direct toxic action on the cellular elements. The briskness of the onset and cessation of sleep led him to adopt Brown-Séquard's view (P) of the onset of sleep as an inhibitory reflex. This is a very reasonable supposition because the onset of sleep which involves firm closing of the eyelids, a rolling of the eyes up and out and a number of other motor adjustments could not be considered a purely *negative* phenomenon, merely a lack of consciousness. But he does not suggest how this reflex is liberated. It is well known that certain conditions such as deliberate preparation for sleep, absence of noise, horizontal posture, voluntary closing of the eyes, and so on, help to induce sleep, but they cannot be considered the actual liberators of the reflex, but as contributory elements only. As to the actual exciting cause of this reflex, opinions vary. Claparède in his "biological" theory of sleep first proposed in 1905 (P) and amplified in 1912 (10) declares that the onset of sleep is due to a reaction of momentary disinterest in one's surroundings. We fall asleep because we are not interested in what is going on around us, but this is facilitated by fatigue and the possible presence of toxic substances in the blood. Claparède did not do any experimental work. According to Shepard (9), "as we go to sleep, we become absorbed in a mass or complex of fatigue sensations. These tend strongly to inhibit other processes, especially motor activity and consciousness of strain sensations from the muscles." While it is true that fatigue will accelerate the onset of sleep, a person can fall asleep when not fatigued at all, and idlers have no difficulty in falling asleep at the usual hour, or at any hour. Pavlov and his co-workers, in their study of the conditioned reflexes, found that their animals frequently fell asleep during the experiment (11), (12). They found that as a result of prolonged action of a uniform excitant their animals invariably fell asleep. The complete data of their experiments have not been published as yet, but in a personal communication to the writer Pavlov states that their results indicate that sleep and the so-called internal inhibition of a conditioned reflex are identical phenomena, the former being diffuse and the latter localized. Another proponent of the uniform or monotonous stimulus effect is Sidis (P) who adds to this limitation of voluntary movements. This is nearer the truth because

an animal usually ceases all voluntary movements when it is going to sleep, and lack of noise or a monotonous sound does help to precipitate sleep. Coriat (8) challenged the work of Sidis, and performed experiments to show that neither limitation of voluntary movement nor a monotonous stimulus are sufficient to induce sleep. He holds that when the subject did not relax his musculature he did not fall asleep under these conditions. Our experiments, while not contradicting Sidis' findings, tend to confirm Coriat's in that relaxation will usually induce sleep under ordinary conditions, but will invariably do so under conditions of prolonged loss of sleep. In our study of basal metabolism under normal conditions the daily determinations were made at 7 a.m. within one hour after the subject got up, and he often fell asleep while lying completely relaxed for 30 minutes. Similar observations were made by Benedict (5) who in describing his new respiration calorimeter warned the prospective users of a tendency of the patient to fall asleep during the test (although instructed to keep awake), and advised gentle tapping on the metal pipe to wake him up. This powerful influence of muscular relaxation was especially striking during our insomnia experiments. All our subjects could keep awake while working or walking or standing, but when under the same external conditions they sat down in a comfortable chair, and especially if they lay down, they fell asleep immediately. Various discordant noises could not prevent the onset of sleep under these conditions, but quiet favored it.

It is now commonly conceded that consciousness is maintained by incoming afferent stimuli. As early as 1876 Heubel (P) working on lower animals came to the conclusion that "mental activity depends on the incoming peripheral sensory stimulations; where such peripheral sensory stimulations are absent, mental activity is in obeyance and sleep results." While we are all aware of visual, auditory and tactile stimuli, few of us are conscious of muscle sense. Indeed, it was not till 1832 that Sir Charles Bell discovered this "sixth" sense, and it was not accepted universally till the end of the 19th century. Due to the numerical preponderance of proprioceptive fibers, the majority of stimuli pouring into the brain come from the muscles, tendons and joints. When a person lies down, the visual sensations soon become monotonous, and muscular relaxation, removing the greater part of the proprioceptive impulses, precipitates what we call sleep. Fatigue favors the relaxation of the sore musculature, and it induces sleepiness by lowering the stream of proprioceptive impulses. Extreme fatigue is known to prevent the onset of sleep, and this will be readily understood if we recall that such fatigue is painful, and painful stimuli pouring into consciousness tend to keep the person awake. Pain is one of the commonest causes of insomnia. All the criticisms advanced against the

vasomotor and intoxication theories, namely, the effect of darkness, silence, horizontal posture, the ability to postpone the onset of sleep in interesting surroundings,—fail in the case of the muscular relaxation theory. This theory also explains the comparatively long sleep of young animals and children, and helps us to understand why people fall asleep amid loud din and racket, if they are so tired that they have to relax their musculature. No one fell asleep during the recent "dancing Marathons." It would be very interesting to study the behavior of blind and deaf persons.

There is one point which all theories of sleep fail to explain. Granted that there is a raising of the threshold for sensory stimulation, how is this brought about? In 1890 Mauthner (P) developed the conception of a blocking of sensory impulses in sleep. Up to date there is no experimental evidence of such blocking, although the idea of increased synaptic resistance is sound. There is some evidence, however, of such an increased synaptic resistance on the motor side. We refer to the positive Babinski sign. An extension of the toes on plantar stimulation during sleep was observed long before the Babinski sign was discovered (by Rosenbach, in 1879; quoted from Piéron). It was subsequently confirmed by Goldflam (P) and Bickel (P). The latter observer found that the reflex became negative on awakening. He also saw a positive Babinski during the early stages of chloroform anesthesia (before all reflexes disappear). The positive Babinski sign, although its nature is not understood, is clinically found in adults only in case of organic pyramidal lesions. In paralysis of hysteria the reflex is negative. It is safe to assume that in the majority of cases it indicates an interruption between the cerebral cortex and the lower motor neurons. However, it was only a year ago that Haberman (2) in a purely theoretical paper on sleep first interpreted the positive Babinski of sleep as does the present writer. Haberman speaks of a cerebrospinal functional break or a shunting off of some part of the cortex. But the rest of his argument is very obscure. If such a switching off of a whole cable can take place on the motor side, why not on the sensory? These functional breaks would not only tend to produce complete muscular rest, but would also prevent impulses from reaching the higher centers and thereby waking the sleeper. This point will be discussed below in connection with the physiology of dreaming. In our experiments every subject showed a positive Babinski during the sleep that followed insomnia, and with remarkable regularity this reflex would be reversed on continued stimulation at very short intervals, confirming the assumption that it was originally due to an *increased synaptic resistance;* this flexion reflex of the big toe obeys the rule for summation of subminimal stimuli, because these stimuli, although strong enough to

elicit the positive Babinski, are too weak to elicit the flexion reflex. The reversal of the Babinski was usually obtained without arousing the sleeper, indicating that the stimuli had not reached the highest centers, but this latter could be accomplished by continued stimulation. On allowing the subject to lapse into slumber after having been awakened, the positive Babinski could be obtained again and again. The same applies to subject N.F. under normal conditions, because he is a deep sleeper.

For the further interpretation of this finding we shall have to pass over to the discussion of the so-called complete theories. We shall not stop to review all the theories based on internal secretions (hypophysis, thyroid), dehydration, exhaustion of intramolecular oxygen, and the various intoxication theories (lactic acid, cholesterol, $CO_2$, leucomains, urotoxins, neurotoxins, etc.). An admirable criticism of all of them can be found in Piéron's book. We may mention that the intramolecular oxygen theory was based mainly on the lowering of the respiratory quotient during sleep. But in the light of modern knowledge, it is clear that this is not due to a storage of oxygen, but to a decreased oxidation of carbohydrates, brought about by muscular relaxation. It is well known that severe muscular exercise will bring the quotient up to unity. The intoxication theories are just as "popular" as are the vasomotor theories, but have the distinction of being "complete" in that they explain at one stroke the cause of sleep, and the mechanism of the onset. It was mainly to test these theories that we undertook the experiments on experimental insomnia, and it is our conclusion that there are no evidences of a general intoxication. During a prolonged abstinence from sleep, there was no change in the $CO_2$ capacity of the blood or the plasma, in the red or white blood count, blood sugar, heart rate, respiration, basal metabolic rate and temperature. After 115 hours of wakefulness the intoxication, if present, was too mild to produce any change in the above and our urinary findings were also negative. Could we then postulate an intoxication at the end of 16 hours of wakefulness? All the arguments brought forward to refute the intoxication idea in the discussion of the partial theories, hold good here. Piéron himself was too cautious to advocate such a theory. What then is the cause of sleep? Among other accepted theories is that of the psychologist, Claparède (10). He decided that sleep was an instinct: "Le sommeil est une fonction de défense, un instinct qui a pour but, en frappant l'animal d'inertie, de l'empêcher de parvenir au stade d'épuisement; ce n'est pas parce que nous sommes intoxiqués, ou épuisés, que nous dormons, mais nous dormons pour ne pas l'être." Such is the power of a phrase that both Sidis and Coriat readily embraced this doctrine in their search for a complete theory of sleep. Yet, Coriat himself remarks that

"any theory of sleep must be based upon sound physiological data, because sleep is a physiological phenomenon." To say that we sleep not because we are intoxicated but in order not to become intoxicated, is no more an explanation of sleep than is to say that we breathe in order not to become intoxicated. As a matter of fact, one can no more commit suicide by refusing to fall asleep than by holding his breath: the reflex will break through in either case. Hunger and thirst have long ago been taken out of the category of "instincts," and put on a sound physiological basis. Nothing will be gained by calling sleep a defensive function. Just as explaining the onset of sleep as being advantageous for the animal at a given moment, Claparède explains waking by saying that it is of greater advantage for it to wake up in the morning than to continue to sleep. In short, there is no "complete" theory of sleep that is in agreement with the majority of facts.

The writer does not claim any originality for the complete theory of sleep he is about to propose. It is merely an attempt to synthesize the many ideas advanced at one time or another by various investigators. As a basis for this theory we shall adopt the doctrine of "levels" in the central nervous system as first advanced by Hughlings Jackson in 1898 (13). He held that "the most complex nervous arrangements, centers and levels are the least organized; the simple, the most organized." A good example of a low level is the respiratory center which is most completely organized and functions in exactly the same manner from birth to death. The highest mental centers are the least organized and are therefore very modifiable, and this enables the animal to learn and establish associations. These highest levels, being the youngest phylogenetically, are much more susceptible to fatigue, intoxication or any kind of injury than are the lower levels. Thus a small dose of alcohol will affect only the highest centers or levels, which exert an inhibitory influence on the natural talkativeness of a person. A larger dose may not only interfere with the ability to make after-dinner speeches, but with his facility in walking properly, and a very large dose is required to paralyze his respiratory center. Likewise in increased intracranial pressure from tumors, granulomata, etc., it is the highest centers that suffer first, and the initial symptoms are described by Osler and McCrae (14) as follows. "The patient may act in an odd unnatural manner, or there may be stupor and heaviness. The patient may be emotional and silly, and there are symptoms resembling hysteria." In our experiments as well as in those of Patrick and Gilbert (P) the only definite effect of insomnia was the depression of the activity of the highest centers that are concerned in analyzing sensations and in solving problems. All our subjects found that they could not study after one night of wakefulness, although they could do ordinary routine

laboratory work fairly successfully throughout the entire period of insomnia. The temporary dissociation of consciousness observed in subject N.K. and in one of Patrick and Gilbert's subjects points in the same direction. It is reasonable to suppose that continued mental activity resulting from wakefulness affects those highest centers first, because they are the least organized and because their metabolism is known to be very active. Whether this fatigue of the higher levels is due to the exhaustion of some nutritive substance in the nerve cells or to an accumulation of the products of cell metabolism cannot be answered at present. On the histological side Piéron reports that he found definitely localized changes in the brains of dogs killed after prolonged periods of sleeplessness, and this indicates that these higher levels have definite localizations in the cortex. In the course of evolution those animals that developed the ability to give these highest centers a rest, could use them to their greatest advantage and won in the struggle for existence. The changes of day and night favored the development of this function. The savages of Central Africa retire with sundown, and speak of nights as so many "sleeps." When it rains and they are forced to remain idle, they sleep. In other words, the periodic cessation of activity due to nightfall (nocturnal animals excepted), coinciding as it did with the end of a day's hunting or fishing, when the animal or primitive man were tired and had to lie down to relax their musculature, gradually developed a switching off of both the afferent and efferent connections of these higher centers, and the phenomenon of sleep was the result. When this procedure was repeated day after day, something in the nature of a conditioned reflex could easily develop, and sleep could thus be set off "precipitously." The highest centers gradually lose their irritability after a day's activity, and this irritability is restored during the night's sleep. Just what happens during sleep is at present unknown, but the results of Campbell and Webster (6) as well as our own show that there is an increased excretion of acids and phosphates in the urine in connection with sleep, whether night or day sleep. However, all this applies to the highest cortical levels whose function is to correctly analyze and interpret the incoming stimuli, to make new associations (learn), and to react in what we call an intelligent manner. But there are lower centers which may not be so fatigued (because of their somewhat better organization), and these may continue to function during sleep, especially light sleep. They are concerned with dreaming. Sensations that are prevented from reaching the highest centers by the increased synaptic resistance, may get to these lower centers, and, while rarely correctly interpreted, start what is known as the dream process. These sensations need not be exteroceptive; they may originate in the viscera. The main characteristic of a dream

is lack of *critical* analysis of events. The dreamer meets people that should be known to him to be in remote regions of the globe, and he is not at all surprised at seeing them. Knight Dunlap (15) in an interesting paper on sleep and dreams speaks of the low integration of dreams as contrasted with the high integration of consciousness. But he holds that the law of association of ideas applies in cases of low integration as well, and this is in agreement with everybody's dreaming experience. Dunlap considers that one of the most characteristic symptoms of disposition to normal sleep is the abolition of the learning process, or of high integration. "Momentary impressions may be made; and a series of perceptions produced: but they do not 'stick'." As an example of the persistence of low integration (our lower "level") he uses a violinist who "may play his part in an orchestra very well, when so sleepy (or so drunk) that he has a very confused notion of what is going on; provided, of course, the composition is very familiar, and played as he is accustomed to play it." This is very much like the behavior of the subject N.K. who dreamt of engaging in a heated argument while he was looking up and calling off correctly logarithms of numbers. We should not have touched the subject of dreaming at all but for the detection of the reversal of the Babinski sign during sleep which seems to show how external or internal stimuli by being frequently repeated will overcome the synaptic resistance and reach these lower levels, producing a dream by low-grade uncritical association of ideas. Should these stimuli become stronger, they will reach the highest levels as well, waking the sleeper. The literature of the subject is full of accounts of how a trivial external happening was misinterpreted by the dreamer and a curious dream built around it. A hot bottle placed under the sleeper's feet may make him dream of walking on the hot lava of Vesuvius. In the absence of external stimuli, internal sensations, hunger, thirst or sexual stimuli may start a dream. Distended seminal vesicles will give rise to an erotic dream, but the powerful stimulus of ejaculation is necessary to overcome all synaptic resistances and waken the sleeper. Generally, however, the dreamer may imagine himself walking about, without actually moving a muscle. The "switching off" of the pyramidal tracts which gives rise to the positive Babinski prevents the efferent impulses from reaching the muscles and thereby protects the sleeper from being aroused by the powerful proprioceptive impulses that would in such event be sent up from the muscles. Sleepwalkers apparently lack the ability to completely shunt off the pyramidal tracts, especially after an exciting evening, and their dreams give rise to actual movements. Why these movements do not wake the sleeper through proprioceptive impulses from the muscles cannot be explained, but in normal individuals this is undoubtedly the case (walking during

a nightmare). This theory also explains adequately a phenomenon which no other theory has been able to elucidate, namely, why the subject, once having fallen asleep, wakes up. It is well known that even in the absence of all external stimuli the sleeper will wake up eventually. Our theory explains this on the basis of internal stimuli of thirst, hunger contractions of stomach, distention of the bladder or rectum, which may become so powerful after some hours of sleep that they overcome all synaptic resistances and reach the highest levels, waking the sleeper.

In this connection a word may be said concerning the periodicity of sleep. Why is it easier to sleep at night than in the daytime, and why will a person accustomed to get up at, say, 6 a.m., wake up at the usual hour, whether he goes to bed early or late? This is a part of the general problem of physiological rhythms which are at present not very well understood, but which are possibly conditioned reflexes. Thus the hunger sensation is periodic; yet it does not appear, in man at least, at definite equal intervals of time, but rather at certain hours, the usual meal hours. Some diurnal rhythms are established early in life, as shown by the activity of the lachrymal glands which ceases at the usual bedtime hour in children ("the advent of the sandman"). Many of the so-called diurnal rhythms are dependent upon sleep for their establishment and maintenance. A good example of this is the temperature rhythm. Benedict (16) found a more or less inverted diurnal temperature curve in a night watchman, but was unable to accomplish this experimentally during an 11 to 12 day period of reversed routine. Toulousse and Piéron (1), however, did succeed in inverting the temperature curve by continuing the reversed routine for 5 or 6 weeks, showing that it was due to sleep, or to the inactivity brought about by diurnal sleep. Our experiments also tend to show (fig. 1) that complete insomnia for several days, involving continuous activity, has the effect of progressively flattening the diurnal temperature curve. Some diurnal rhythms can be inverted much sooner, as shown by the change in the secretory activity of the kidney on a reversed routine.

In conclusion the writer wants to state that this theory is provisional and is to be used only as a working hypothesis. As Piéron says: *Une théorie n'est pas la solution d'un problème, c'est contraire l'énoncé d'un problème à résoudre.*

## SUMMARY

1. Experiments were performed to study the effects of experimental insomnia in man, the duration of complete sleeplessness being from 40 to 115 hours.

2. Subjectively the persons employed could easily keep awake while engaged in some sort of activity, but felt very drowsy when sitting, and fell asleep immediately on lying down.

3. Muscular relaxation induces sleep under normal conditions (confirming Coriat), but practically precipitates sleep under conditions of experimental insomnia.

4. Blood sugar, alkaline reserve of the blood and plasma, percentage of hemoglobin, percentage of corpuscles, red and white blood cell count, body weight, basal metabolic rate, appetite, temperature, ability to name letters and to do mental arithmetic, all of these showed no variations from normal during the period of sleeplessness.

5. Respiration, heart rate, blood pressure showed a marked decrease in insomnia, but this decrease was mainly due to greater muscular relaxation of the sleepy subject.

6. Numerous reflexes were found to be present in sleep following insomnia, but the response was somewhat sluggish. Sleep seemed to be deeper six hours after its onset than at the end of two hours.

7. A positive Babinski reflex could be elicited in every subject tested during the sleep that followed insomnia. It is interpreted as indicating a functional block of the pyramidal system of fibers.

8. This reflex could be reversed by rapidly repeated stimulation of the sole. The depth of the sleep decreased at the same time. This indicates that a number of subminimal stimuli overcome the synaptic resistance and produce a flexion of the great toe.

9. There is a greater excretion of phosphates and acids at night; but on reversed routine, with the subject sleeping in the daytime, this condition is reversed, indicating that the increased excretion is due to sleep (confirming Campbell and Webster).

10. There is a greater excretion of chlorides in the daytime; the same is true in insomnia; but there is a tendency to reversal in the subject that sleeps during the day.

11. The excretion of total nitrogen and of creatinine shows little diurnal variation, and is unaffected by either insomnia or reversed routine.

12. There is some evidence that the diurnal temperature variation is due to the alternation of sleep and wakefulness, and the temperature wave tends to be effaced during prolonged insomnia.

13. The onset of sleep is probably due to complete muscular relaxation, voluntary or involuntary.

14. A provisional theory is proposed based on the conception of "levels" in the central nervous system, as first elaborated by Hughlings Jackson. Sleep may be due to fatigue of the highest centers of consciousness, and dreaming to the persistence of the activity of the lower centers.

I wish to express my thanks to my colleagues in the laboratory for the help they rendered me in this work. Especial thanks are due to Mr. Charles C. Adams and Mr. Mark T. Phy whose genuine interest and splendid loyalty made this research possible. I shall always cherish the memory of the many sleepless nights we spent together.

I am greatly indebted to Dr. A. J. Carlson for the many helpful suggestions and criticisms in the course of the work, and for the revision of the manuscript.

## BIBLIOGRAPHY

(1) Piéron: Le Problème Physiologique du Sommeil, Paris, 1913.
(2) Haberman: Med. record, 1922, ci, 265.
(3) Smith: Brit. Journ. Psychol., 1916, viii, 327.
(4) Robinson and Herrmann: Journ. Exper. Psychol., 1922, v, 19.
(5) Benedict: Boston Med. and Surg. Journ., 1918, clxxviii, 667.
(6) Campbell and Webster: Biochem. Journ., 1921, xv, 660; 1922, xvi, 507.
(7) Kunde: This Journal, 1923, lxiii, 401.
(8) Coriat: Journ. Abnorm. Psychol., 1912, vi, 329; 1912, vii, 94.
(9) Shepard: The circulation and sleep, New York, 1914.
(10) Claparède: L'année Psychologique, 1912, 419.
(11) Petrov: Compt. Rend. Soc. Biol., 1916, lxxix, 1067.
(12) Pavlov and Voskressensky: Compt. Rend. Soc. Biol., 1916, lxxix, 1079.
(13) Jackson: Brit. Med. Journ., 1898, i, 65.
(14) Osler and McCrae: The principles and practice of medicine, New York, 1921, 1005.
(15) Dunlap: Journ. Abnorm. Psychol., 1921, xvi, 197.
(16) Benedict: This Journal, 1904, xi, 145.

# THE CALORIGENIC ACTION OF ADRENALIN CHLORID[1]

WALTER M. BOOTHBY AND IRENE SANDIFORD

*From the Section on Clinical Metabolism, Mayo Clinic, and Division of Experimental Surgery and Pathology, The Mayo Foundation, Rochester, Minnesota*

Received for publication April 10, 1923

The studies on the effect of adrenalin on the metabolism, as evidenced by alteration in the quantity and character of the gaseous or respiratory exchange, were initiated by Barcroft and Dixon in 1906. By means of perfusion from a living hirudinized animal they maintained a circulation of undiluted blood through the coronary system of an excised heart, and determined the carbon dioxide and oxygen tensions of the blood. Barcroft and Dixon demonstrated that after the addition of adrenalin to the blood the oxygen consumption was increased two to three times, while the carbon dioxide elimination lagged behind the changes in the activity of the heart They interpreted the changes in oxygen consumption as due to changes in tonus as well as in amplitude of beat.

La Franca, in 1909, reported an increase in the total gaseous exchanges, but no alteration in the respiratory quotient from the injection of adrenalin.

Bernstein and Falta, in 1912, found that in man 1 mgm. of adrenalin injected subcutaneously resulted in a rapid increase in oxygen consumption which reached its height one hour after the injection; they attributed the reaction to increase in tonus of the sympathetic nerve endings. Fuchs and Roth likewise found increases in oxygen consumption of 34 to 63 per cent in human subjects from 1 mgm. of adrenalin injected subcutaneously; they presented no explanation of the increased oxygen consumption, but interpreted the rise in the respiratory quotient as due to increased combustion of sugar. The next year, however, they failed to confirm the rise in respiratory quotient and therefore retracted their previous suggestion of increased carbohydrate combustion; in neither paper is serious attention given to the increase in the metabolism.

[1] Prof. Graham Lusk has requested that the term "specific dynamic action," as used in our original reports to indicate the calorific or heat producing power of thyroxin or adrenalin, be reserved to express the increase in heat production following the ingestion of foodstuffs, maintaining the sense of that term as originated by Rubner. The term "calorigenic action" which we have adopted was suggested to us by Professor Lusk, after consultation with Dr. S. R. Benedict, to signify the specific stimulus to metabolism given by such substances as thyroxin and adrenalin.

Hari, in 1912, using curarized dogs, failed to find any increase in oxygen consumption after either intravenous or intraperitoneal administration of 0.5 to 2 mgm. of adrenalin for each kilogram of body weight; in fact, he obtained a decrease in both carbon dioxide and oxygen although there was a slight elevation of the respiratory quotient. Hari refers to a report made by Welecki before the Cracow Academy of Science in 1909, in which the latter asserts that after an intraperitoneal injection of adrenalin there is a decrease in the carbon dioxide production, and after an intravenous injection of adrenalin, an increase. Wilenko, in 1912, on the other hand found in normal rabbits under urethane anesthesia that the respiratory quotient, after oral or subcutaneous administration of glucose, rose to over 0.88; however, in five experiments with glucose and adrenalin there was practically no change in the respiratory quotient and Wilenko therefore concluded that adrenalin decreases the ability of the body to burn sugar.

Patterson and Starling, in 1913, demonstrated that adrenalin increases the consumption of glucose in perfusion experiments on the heart, by showing that the glucose disappeared much more quickly from the circulating blood when adrenalin chlorid was added to the solution than when not. They believed this increased consumption of sugar was due to a specific effect of adrenalin on sugar combustion, in agreement with Evans who, in 1912, had shown that the total metabolism of the heart is approximately proportional to its rate, and that the latter is very largely increased by the administration of adrenalin.

Evans and Ogawa, in an excellent paper published in 1914, demonstrated that the oxygen consumption of an isolated mammalian heart was approximately doubled by introducing 1 cc. of a 1:10,000 solution of adrenalin into the circulating fluid resulting in a concentration of about 1:3,000,000. The respiratory quotient varied, usually falling and then rising markedly, this being interpreted by Evans and Ogawa as being due to a lag in the completion of the reaction. They believe that adrenalin neither increases nor decreases the power of the tissues to utilize carbohydrate, and they were not particularly impressed with the increased oxygen consumption, apparently because in the heart this might be accounted for by increased muscular activity and greater work.

Lusk and Riche, in 1914, using larger doses of adrenalin (10 to 11 cc. of 1:1000) with and without the ingestion of 50 grams of glucose, obtained a respiratory quotient approaching 0.9 and a marked increase in heat production, but no increase in protein metabolism. As the animal became so restless after the injection of adrenalin, the investigators did not feel justified in considering the increase in heat production of much physiologic significance. However, the fact that the

respiratory quotient materially rose, together with a marked increase in sugar consumption, following administration of adrenalin either with or without previous ingestion of glucose, led them to conclude that the elaborate theory promulgated by Falta, Eppinger and Rudinger and supported by Von Noorden is untenable in any of its particulars; namely that epinephrin causes a production of sugar from fat, decreases the power of the organism to oxidize glucose through inhibition of pancreatic function, and stimulates the thyroid so that protein metabolism is increased.

Anderes and Cloetta, in 1916, demonstrated that an increase in oxygen absorption following adrenalin injection is accompanied by an increase in volume of blood passing through the lungs. Jackson, in 1916, reported one experiment which he thought might indicate a decrease in the oxygen consumption during the few minutes following an intravenous injection of 0.5 mgm. of adrenalin.

Peabody, Clough, Sturgis, Wearn and Tompkins, in 1918, in a preliminary report, showed that following the intramuscular injection of adrenalin there was usually an increase in the metabolism independent of the so-called Goetsch reaction and accompanied by an increase in the blood sugar. The detailed report of these experiments by Tompkins, Sturgis and Wearn revealed that the intramuscular injection of 0.5 cc. of 1:1000 adrenalin causes an increase in the metabolic rate and in the respiratory quotient not only in normal men, but also in patients with "irritable heart" and in hyperthyroidism. They concluded that adrenalin acts indirectly causing an increase in metabolism through the sympathetic system by producing changes in tremor, hyperglycemia and so forth, which they consider as probably the actual cause of the increased heat output; the question of adrenalin acting as a direct stimulant to cellular metabolism was only mentioned to be dismissed as improbable. As late as 1921 Peabody, under whose direction the above work was done, said "On the basis of what is definitely understood with regard to the physiologic action of epinephrin it seems probable that the phenomenon is due to a stimulation of the sympathetic nervous system"; he does not consider the possibility of a definite and direct calorigenic action on cellular metabolism.

Sandiford, in June, 1919, completed a series of experiments similar to those of Peabody and his associates, but a record of Sandiford's experiments was not published in detail until 1920. A summary, however, was presented by Boothby and Sandiford at the meeting of the American Physiological Society in December, 1919, as follows: It was found that adrenalin invariably caused an increase in the heat production, and in thirty-nine of forty-six experiments there was an increase in the respiratory quotient. This rise in heat production is accompanied by

An increase in the ventilation rate, and as a rule by an increase in the respiration rate, number of heart beats each minute, volume of each beat, greater utilization of the blood-carrying power, and peripheral vascular dilatation with an increased systolic and decreased diastolic blood pressure. Not all these compensatory factors necessarily come into play in each instance. As would be expected, various combinations may occur, sometimes one factor, sometimes another, acting as the major compensation. The data show that there is no relationship between the character of the adrenalin reaction and the degree of activity of the thyroid gland, and therefore, Boothby and Sandiford believe, contrary to the opinion of Goetsch, that the reaction is not indicative of the presence or absence of hyperthyroidism The similarity of the metabolic rate curve following the injection of adrenalin to that found by Lusk from carbohydrate plethora, naturally directs attention to the possibility that the increased heat production is due to an excess of carbohydrate metabolites. Boothby and Sandiford concluded, however, that there might also be a direct chemical stimulation of cellular combustion.

Marine and Lenhart, in 1920, using large doses of adrenalin (0.5 cc. for 1 kgm.) confirmed these observations by showing a rise in oxygen consumption in normal and in thyroidectomized rabbits. As a rule the absolute rise was greater in normals while the percentile rise was usually unaltered.

Boothby and Sandiford, at the meeting of the American Physiological Society, December, 1920, presented direct evidence that the calorigenic action of adrenalin can not be interpreted as the simple effect of a carbohydrate plethora. Their evidence consisted of forty-one experiments on human subjects in which the increase in the heat production, respiratory quotient and blood sugar concentration from the ingestion of 100 grams of glucose was compared with the results obtained in twenty-two experiments following the subcutaneous injection of 0.5 mgm. adrenalin; these experiments, summarized in table 1, showed that from the ingestion of glucose there was an increase of 9 per cent in the heat production for an increase in the blood sugar of 105 per cent, while from the injection of adrenalin there was an increase of 20 per cent in the heat production for an increase of only 37 per cent in the blood sugar concentration, with a correspondingly less increase in the respiratory quotient. They also reported one experiment in an essentially complete diabetic patient with hyperthyroidism in whom they obtained a very pronounced rise in heat production after the administration of adrenalin, but found practically no increase in heat production from the administration of glucose although there was a very marked

increase in the blood sugar concentration. The details (previously unpublished) of this experiment are given in table 2, and show that after the ingestion of glucose there is no evidence in the curves of the metabolic rate or of the respiratory quotient, that there was any combustion of sugar, or any calorigenic effect from the high level of carbohydrate metabolites in the blood; in contrast there is shown a marked effect on the metabolic rate from the injection of adrenalin with only a slight increase in the blood sugar.

The dose of adrenalin used in these experiments on human subjects was 0.5 mgm., which amounted for an average individual of 70 kgm. to approximately 0.007 mgm. for each kilogram; Rogoff in the discussion of the report pointed out that this dose was apparently considerably in

TABLE 1

*The average maximum effect on the heat production, respiratory quotient and blood sugar in forty-one experiments after the ingestion of 100 grams glucose, and in twenty-two experiments after the subcutaneous injection of 0.5 mgm. adrenalin*

| EXPERIMENTS | | GLUCOSE | ADRENALIN |
|---|---|---|---|
| Basal metabolic rate | Before | +44 | +34 |
| | After | +57 | +62 |
| | Increase | 13 | 28 |
| Calories, per cent increase | | 9 | 20 |
| Blood sugar | Before | 0.132 | 0.143 |
| | After | 0.271 | 0.196 |
| | Increase | 0.139 | 0 053 |
| | Per cent increase | 105.0 | 37.0 |
| Respiratory quotient | Before | 0.77 | 0.77 |
| | After | 0.93 | 0.87 |
| | Increase | 0.16 | 0.10 |

excess of the ability of the adrenals to secrete, according to the quantitative data advanced by Stewart and Rogoff as to the probable physiologic output. However, as the injection was given subcutaneously, and as it is well known that absorption under such circumstances is not instantaneous but takes considerable time, it seems unlikely that this would be completed in less than ten minutes; therefore if, for instance, the maximal rate of absorption is assumed to be twice this (complete absorption in five minutes) or at the rate of 0.0014 mgm. each minute, then the dose must be considered as being of the order of magnitude at least comparable with that which they found possible for the adrenal glands to secrete. Even if it were assumed that the adrenalin was entirely absorbed in as short a time as two minutes, then

the dose for each kilogram each minute would still be less than 0.0035 mgm., or within the upper limits of excretion found by Cannon.

At the same meeting, in December, 1920, Aub, Forman and Bright reported that in three adrenalectomized cats they found, first a slight rise for some hours in the metabolic rate, followed forty-eight hours later by a sharp fall to about 25 per cent below the normal, while in control operations the metabolism fell only 12 per cent. On the other hand, Marine and Baumann reported that in rabbits after partial injury

TABLE 2

*Effect on metabolic rate, blood sugar, and so forth, of ingestion of 100 grams glucose, and of subcutaneous injection of 0.5 cc. adrenalin*

Case A149225, Mrs. M. R., aged forty-five years.
Diagnosis: exophthalmic goiter, diabetes mellitus.

| DATE, 1920 | BASAL METABOLIC RATE | RESPIRATORY QUOTIENT | PULSE | RESPIRATION | TEST* | BLOOD SUGAR | REMARKS |
|---|---|---|---|---|---|---|---|
| | | | | | | *mgm.* | |
| 9–24 | +91 | 0.68 | 126 | 26 | S | 259 | Basal |
| | +99 | 0.69 | 144 | 22 | S | 356 | One-half hour after ingestion of 100 grams glucose |
| | +88 | 0.70 | 155 | 24 | S | 440 | One hour after ingestion of 100 grams glucose |
| | +88 | 0.70 | 141 | 25 | S | 438 | Two hours after ingestion of 100 grams glucose |
| | +80 | 0.68 | 140 | 19 | S | 390 | Three hours after ingestion of 100 grams glucose |
| 9–30 | +78 | 0.68 | 147 | 26 | S | 336 | Basal |
| | +149 | 0.75† | 190 | 31 | FS | 374 | Ten minutes after injection subcutaneously of 0.5 cc. adrenalin |
| | +106 | 0.72 | 185 | 31 | S | 359 | Thirty minutes after injection |
| | +88 | 0.61 | 165 | 26 | S | 331 | One hour after injection |
| | +91 | 0.63 | 165 | 25 | S | 356 | One and one-half hours after injection |

* S, indicates that the test was satisfactory, and FS that it was only fairly satisfactory.

† This increase in respiratory quotient is not necessarily due to an increase in carbohydrate combustion. More likely it was due to the washing out of carbon dioxid by the increased respiration; this view is strengthened by the following low quotients caused presumably by reaccumulation of carbon dioxid. The subjective and objective reaction from adrenalin was very marked, and it was thought unwise to repeat the experiment under similar conditions. The patient moved slightly and was restless in the period immediately after the injection, but was quiet in the following periods. The increase in metabolism, however, was very marked and exceeded the probable error due to the subjective discomfort and the slight movements.

to the adrenals there was an increase in heat production. In their complete paper appearing shortly afterward Marine and Baumann reported forty-two experiments in rabbits, in which the adrenals were removed or injured by freezing and, as a result, the metabolism did not change in 33 per cent, a primary increase was followed by a fall to, or below, normal in 57 per cent, and there was a rapid fall with death in 9 per cent. In their discussion these investigators emphasized the importance of the primary rise which occurred in more than half their experiments, and even suggested that the reaction in these animals resembled the syndrome of exophthalmic goiter. They assumed that the operative procedure of extirpation and injury by freezing produced, in both instances, an insufficiency of epinephrin, and therefore were of equal value in the determination of the effect on the metabolism of a decreased or absent epinephrin secretion.

In June, 1921, Aub and Bright presented before the Association for the Study of Internal Secretions a preliminary report of the work published in 1922 on the effect on the metabolic rate of the removal of the adrenal glands in urethanized cats. They found that even when the blood pressure and the body temperature were kept normal after the operation, the metabolism dropped sharply a few hours later. By control experiments they demonstrated that this decrease was apparently not the result of the surgical procedures or manipulation. They pointed out that the reduction in the metabolism in the acute experiments following adrenalectomy with the cats still under urethane anesthesia was much more rapid than that obtained on animals operated on under ether anesthesia and on which the metabolism was determined without anesthesia on succeeding days after operative recovery. Since the decreased metabolic rate was brought back to normal by the injection of adrenalin at the rate of 0.004 mgm. each minute, it was concluded that the loss of the adrenal medulla was the dominant factor in causing the decreased metabolism, although experiments designed to exclude the effect of the removal of the cortex were inconclusive. In our opinion a very significant part of these experiments was the evidence that in the absence of the adrenal glands a small dose of adrenalin, administered intravenously at a rate approximately equivalent to the outside physiologic limits of the adrenal glands to secrete, did elevate the metabolic rate; thus these experiments give direct experimental evidence of the validity of the assumption made in our earlier work on human subjects that the dose of 0.5 mgm. adrenalin injected subcutaneously was absorbed at a rate at least roughly within the limits of the possible physiologic output of the adrenal glands.

Marine and Baumann, in 1922, reported that they found in a series of six experiments on rabbits that thyroidectomy caused a distinct

decrease in metabolism amounting to from 13 to 31 per cent, and that subsequent complete adrenalectomy caused a still further drop of varying magnitude. In their discussion they stress the fact that, unlike their previous experiments in rabbits with intact thyroids, the metabolism did not show a primary increase; they therefore concluded that the difference in the reaction indicates a thyroid-adrenal interrelationship which may have a bearing on the etiology of exophthalmic goiter. We believe, however, that further experiments will be necessary before it can be accepted as an established fact that the primary rise in metabolism noted by Marine and Baumann as sometimes occurring after adrenalectomy, is to be considered the result of adrenal insufficiency, or that the mechanism of the reaction is through a hypothetical relationship of the adrenal glands to the thyroid gland.

Our third report, made at the meeting of the American Physiological Society in December, 1921, was based on experiments made on dogs under urethane anesthesia. Evidence was obtained of a definite calorigenic effect from the intravenous administration of adrenalin at a rate as low as 0.0045 mgm. for each kilogram each minute. The technical difficulties associated with the production of anesthesia and the complicating influence of the urethane, prevented an accurate estimation of the effect of a lower rate of administration. These experiments, like those of Aub and Bright, were still open to the criticism that the dose used was only within the estimated maximal value of secretion found by Cannon, and was in excess of the values found by Stewart and Rogoff. At this same meeting, Martin and Armitstead presented evidence to indicate that adrenalin increases the resting metabolism of the isolated sartorius muscle of the frog, as measured by the development of acidity. In two papers presented in 1922, they give in detail their evidence that adrenalin in concentrations as low as 1:200,000 increases the metabolism of isolated tissues two to threefold. Garrey has likewise shown a similar increase produced by adrenalin on the respiratory processes and automatic impulse formation of the cardiac ganglion of *Limulus polyphemus*. Hutchinson, however, failed to confirm the results of Martin and Armitstead.

Griffith, however, working in Cannon's laboratory, has recently reported similar experiments with an improved form of the Osterhout respiration apparatus. He shows that by this method, and particularly by controlling such factors as the pH and the buffer action of the solutions used, that adrenalin has no effect upon the carbon dioxid production of surviving frog muscles. He likewise showed that adrenalin has no effect upon the total acid production of isolated skeletal

muscles of the frog. Griffith specifically points out, however, that his results do not permit a criticism of experiments in which adrenalin increases the metabolism of cats and man (similar to those in this paper on dogs) nor does he consider that any conclusions can be drawn from his experiments on the relationship of the adrenal glands to metabolism.

Aub and Taylor, in their review of the literature in March, 1922, briefly refer to the experiments of Aub, Bright and Uridil, at that time unpublished, which demonstrated that the metabolic effects of the thyroid and adrenal glands may be independent. In his excellent presentation of the theoretic side of the subject at the symposium on Endocrinology at the 1922 meeting of the American Medical Association, Aub clearly emphasized that the evidence so far accumulated indicates that the mechanisms of the calorigenic action of adrenalin and of thyroxin (and by inference each gland respectively) are independent one from the other. He pointed out that this interpretation is greatly strengthened by the time relationship of the two reactions as shown by Plummer and Boothby for thyroxin and by Boothby and Sandiford for adrenalin. Boothby called attention to the fact that the curve of the calorigenic effect of adrenalin is quite similar to that of thyroxin, provided that the time element of the first is charted in units of minutes and of the latter in days.

Scott, in 1922, reported a series of thirty-five experiments on twenty-four cats, in which a variable effect on the metabolism was produced by freezing, ligations and partial removal of the adrenal glands. Six experiments are noted in detail, illustrating groups which showed increase, decrease or no change in the heat production following these procedures. As the result of freezing, a temporary rise in heat production was obtained in six of ten experiments (60 per cent); the effect of ligation showed nearly equal numbers in each group; in 88 per cent there was no change in the metabolism from partial removal and trauma. Scott concluded from these data, however, that severe and sufficient non-fatal injury to the suprarenal cortex by freezing or ligation in cats causes a significant and prolonged increase in heat production, while insufficient injury causes no significant alteration and that lethal injury causes a decrease.

Marine and Baumann, in 1922, reported a series of nine experiments which demonstrate a decrease in heat production in rabbits after thyroidectomy, followed, as a rule, by further decrease after adrenalectomy; they again emphasize their previous evidence that the primary increase in heat production after adrenalectomy, often found by them in rabbits with the thyroid intact, does not occur if the thyroid has been removed. In another report they present fur-

ther evidence to support their previous observation that partial, but sufficient destruction of the suprarenal cortex in rabbits with intact thyroids, causes a prolonged rise in heat production; in all they find a prolonged absolute rise in heat production in 82 per cent of thirty-three rabbits in which both suprarenal glands had been completely removed, and in 40 per cent of fifteen rabbits in which the suprarenal function was crippled by freezing. The period of increased heat production may last from a few days to several months. They point out, however, that these rabbits were not in the post-absorptive state.

The experimental data of Aub and his associates referred to above, as they appeared in the various Society communications have been presented in detail, together with an analytical review of the pertinent literature on the subject, in a series of three splendid papers in the July, 1922, issue of the AMERICAN JOURNAL OF PHYSIOLOGY. In the first paper are grouped the experiments of Aub, Bright and Uridil, which show that the increased basal metabolism stimulated by thyroxin cannot be explained by muscular activity, muscular fibrillation or increased muscle tonus; and it is also proved that the adrenal glands are not essential to the maintenance of the high metabolic rate induced by thyroxin. In the second paper Aub, Forman and Bright report four experiments in which they found that the metabolism fell about 25 per cent below the average normal forty-eight hours after double adrenalectomy had been performed, and decreased until death on the fourth or fifth days. Control experiments on completely fasting cats operated on in a similar manner but without removal of the adrenal glands showed a fall in metabolism less than half the magnitude of that seen after adrenalectomy. Although the small number of experiments and the technical difficulties attending these experiments prevent their acceptance as absolute proof, yet the care and attention to details with which they have been carried out render it extremely probably that a characteristic decrease in metabolism develops a few days after complete adrenalectomy in cats, and gradually decreases until death. In the third paper, Aub, Bright and Forman report twenty-four experiments on the effect of adrenalectomy and various control procedures on the basal metabolism of the urethanized cat. These are all acute experiments lasting less than five hours from the time of complete anesthetization to the end of the observations, including operation and other procedures. The expired air was collected by a tracheal cannula in a gasometer and analyzed in duplicate. These experiments show, as is pointed out, a prompt and progressive fall of the metabolism after removal of the adrenal glands with the cat under

urethane; this decrease in a few hours averages 12 per cent, and sometimes it is as large as 28 per cent. On account of the rapid fall in the metabolic rate, unlike the slow decrease shown in the second paper, the authors are obliged to assume that the urethane was a contributory factor and that it emphasized the symptoms of adrenal insufficiency; the evidence cited from the literature to substantiate this assumption is not in itself sufficiently established to be of much weight.

EXPERIMENTAL EVIDENCE OF THE EFFECT IN DOGS OF THE INTRAVENOUS INJECTION OF ADRENALIN AT A RATE BETWEEN 0.0006 MGM. AND 0.0025 MGM. EACH KILOGRAM EACH MINUTE. The present series of experiments, briefly reported to the American Physiological Society in December, 1922, was carried out according to the metabolism technic fully described in our laboratory manual. All readings of the gasometer, time, temperature, barometer, and so forth, were made by two persons; the analyses of the expired air were made in duplicate, and in the crucial experiments in triplicate. Only those points of the technic which were necessarily modified to render the gasometer method applicable to dogs will be described here in detail.

Care was exercised to select dogs of a quiet and gentle disposition; if, after trial, they could not be readily trained, they were discarded. As soon as selected, the dogs were placed on an approximately constant diet of about 18 to 20 grams for each kilogram of body weight of Champion dog biscuit no. 5 (protein 22 per cent; fat 4 per cent; carbohydrate 61 per cent). The dogs were fed in the afternoon between 3 and 5 o'clock, thus allowing not less than sixteen hours to elapse between the ingestion of food and the starting of the experiment. Several days were spent in training a dog to lie quietly for several hours on the laboratory table, first without the mask, and later with the mask adjusted. After placing the dog on the table and adjusting the mask, a preliminary period of at least one hour is necessary before it can be assumed that the metabolism has quieted down to a basal level; even with this delay it can be seen from the curves that the second normal period tended to be slightly lower. Throughout the entire experiment including the preliminary period, quiet was maintained in the room so that the dog was not disturbed. It was impossible to maintain the room at the same temperature on different days; if the room was cool, the dog was covered with a blanket to prevent shivering. Lusk maintained a constant environmental temperature because his dogs were enclosed in a respiration calorimeter, and in contrast our experiments may be somewhat affected thereby, as Rubner has shown that in the dog the basal metabolism can be materially influenced by marked changes in the envi-

ronmental temperature. Each metabolism period was approximately thirteen to fifteen minutes in duration.

A copper mask[2] with outlet and inlet tubes was used, of such a size that it would readily fit over the dog's head and extend to the region of the ears, corresponding to the largest diameter of the head. The eyes and ears being thus covered, there was less chance of the dog's being disturbed by outside influences. The mask was held in place by tapes which passed around the neck and back of the ears like a loose collar; under ordinary circumstances there was no tension on these tapes and their main purpose was to secure the mask during the training period in case of sudden movements. A 5-inch roll rubber dental dam was fastened by one or two turns around the copper mask[2] and then carried down in smooth windings over the closely shaved neck of the dog, and finally brought back over the mask and after a few turns fastened. The rubber bandage must not be so tight as to cause constriction of the neck and discomfort, or to cause wrinkling and folding of the skin, thereby making channels through which a leak might occur, and the bandage must not be applied so loosely that an air-tight fit is not obtained. Both extremes can be readily overcome by care and practice. Smearing the neck and rubber bandage with vaseline has proved, as a rule, unnecessary. At the end of the experiment and before the mask was taken off, its application was tested for tightness by weighting the spirometer and thus allowing the dog to breathe into the spirometer under pressure, and applying soap suds to rubber bandage and neck. Toward the end of the study we improved the method of testing the mask by disconnecting the expiratory tube from the gasometer connection and immersing it into a large bottle of water for a distance of from 15 to 20 cm., thus increasing to a greater degree the positive pressure and rendering it easier to detect leaks.

To record muscular movements two pneumographs were adjusted; the first passed over the upper part of the chest just behind the foreleg in such a manner that the slightest movements of the forelegs or head were indicated on the tracing, and the second passed over the lower part of the trunk just in front of the hind leg so that movements of the hind legs as well as those of the tail were recorded. The value of such tracings is to record graphically for future reference the presence or absence of any material extraneous muscular contractions, as well as the rate and character of the respirations; the arrangement

[2] The outside surface of the copper mask must be smooth and even without a ridge at the juncture of the copper sheet where it folds over to complete the cylinder. If the fold is on the outside, the ridge forms a channel underneath the rubber bandage through which air may leak out.

is exceedingly delicate and records minute extraneous movements, if such occur, and for our purpose gives a somewhat better idea of their relative magnitude than the device used by Benedict in the respiration calorimeter. The occasional involuntary twitching of the legs which is so common in dogs lying at rest caused us some anxiety at first, but it was found that, unless very frequent, no demonstrable elevation of the metabolism resulted. We had no means of knowing whether the dog was awake or asleep and it is not improbable that some of our lowest basal observations represent the depression produced by sleep. Carpenter has emphasized the fact that sleep may depress the metabolism 5 to 10 per cent or even more, and also cause variations in the respiratory quotient.

The urinary nitrogen was not determined and the original Zuntz and Schumberg table for the calorific value of oxygen was used in the computations without allowance for protein metabolism. No appreciable error is introduced by this method and, in fact, it is probably just as accurate for short period experiments as the more complicated method, on account of the error introduced in the latter by the lag in the nitrogen elimination.

The technical experimental error is the summation of many accidental positive and negative errors. In the determination of the volume of the expired air the linear rise of the gasometer is recorded to the nearest 0.05 cm. The volume of the expired air is, therefore, determined with an accuracy in the gasometer used of 63 cc. and as the experimental volume is 30 liters or more, the error is at most 0.2 per cent. The temperature of the air volume in the gasometer is read from a thermometer inserted about 10 cm. through the top of the bell and is recorded to the nearest 0.2°C. The assumption that the air in the gasometer is completely saturated at the temperature observed at the top of the bell is in accordance with general practice; as the expired air leaving the lungs is nearly saturated at 37°C., it seems safe to assume complete saturation at room temperature. The barometer correction is computed on the basis of the nearest millimeter. The analyses are carried out in duplicate or triplicate and are accepted only if within $\pm 0.03$ per cent of the average for oxygen and $\pm 0.02$ per cent for carbon dioxid, and the routine outdoor air analyses must also agree within these limits; the composition of the inspired air is taken as 0.04 per cent for carbon dioxid and 20.93 per cent for oxygen. Stratification of the air in the gasometer may cause an error in the determination of the average composition of the magnitude of $\pm$ 0.06 per cent, which variation, however, includes the smaller analytical error mentioned. The sum of all these errors cannot exceed 0.5 per cent in the final results and is usually very much less.

The technical errors, therefore, are well within the limits of the physiologic requirements, because under even the best experimental conditions the absolute basal metabolism cannot always be obtained. For example, in dog F257, on which were obtained sixty-nine satisfactory normal basal periods (omitting the four periods of March 13th, which were the first of the series, as well as tests which were not satisfactory, indicated by *F* on the charts), the average total calories was 27.1. The average variation, regardless of sign, was 4.8 per cent although in one instance there was an increase above the average of 17 per cent in the third basal period of experiment XVI on May 10th. This large variation may possibly be accounted for by the fact that on each of the two preceding days the dog had undergone long experiments and may not have been feeling perfectly fit. The variations of the individual normal periods on dog F754 are of less magnitude; the maximum is 23.4 calories (10 per cent) above the grand average of 21.2 calories and the minimum is 18.1 calories (15 per cent) below this average; the average variation of each period from the grand average is 0.8 calorie, less than 4 per cent.

In comparing our individual basal periods with those reported by Lusk it must be remembered that our periods are of short duration, ten to fifteen minutes, while Lusk's are hourly periods which would be equivalent to an average of four or five of our periods. With this in mind we averaged the two or three basal periods for each day, totaling thirty to forty-five minutes to see if the base line became more constant. The basal total calories for each period, averaged for each day with the percentage variation from the grand average, are given for dog F754 in table 3, and demonstrate a very satisfactory constancy from day to day although there was a quite gradual progressive decrease, most marked during the last of the series. As mentioned, some of our lowest basal periods may be due to the dog dropping off to sleep, and some of the highest to temporary discomfort or to a lowering of the environmental temperature. The order of magnitude of the elevation of metabolism as a result of the injection of adrenalin is, however, sufficiently great, as may be readily seen from the curves, to exceed the combined physiologic and technical errors inherent in the method.

The errors of analyses and stratification of the air in the gasometer may cause an error in the respiratory quotient of the order of $\pm 0.01$. The magnitude of the error in the respiratory quotient from alteration in the character of the respiration either spontaneously or as a result of the injection of the adrenalin cannot at the present time be evaluated with accuracy, although there is no reasonable doubt but that, on the average, they represent within $\pm 0.02$ the true combustion

quotient, although any individual quotient may theoretically, through forced breathing, be in error by as much as ±0.1.

TABLE 3

*Basal metabolism periods on dog F754*

The forty-five satisfactory periods averaged 21.2 calories (experiments enclosed in brackets omitted from the average)

| DATE, 1922 | TOTAL BASAL CALORIES | VARIATION EACH PERIOD FROM GRAND AVERAGE OF 21.2 CALORIES | DAILY AVERAGE | VARIATION OF DAILY AVERAGE FROM GRAND AVERAGE OF 21.2 CALORIES |
|---|---|---|---|---|
| | | *per cent* | *calories* | *per cent* |
| 10–12 | (24.8) | | | |
| | (24.5) | | | |
| | (23.8) | | | |
| 10–13 | 22.5 | +6 | | |
| | 22.2 | +5 | | |
| | (22.2) | | 22.4 | +6 |
| 10–16 | 22.3 | +5 | | |
| | 21.6 | +2 | | |
| | 23.4 | +10 | 22.4 | +6 |
| 10–20 | 22.1 | +4 | | |
| | (23.9) | | | |
| | 22.6 | +7 | 22.4 | +6 |
| 10–24 | 22.5 | +6 | | |
| | 21.7 | +2 | | |
| | 21.9 | +3 | 22.0 | +4 |
| 10–26 | 21.9 | +3 | | |
| | 21.4 | +1 | | |
| | 21.4 | +1 | 21.6 | +2 |
| 10–28 | 21.6 | +2 | | |
| | 22.2 | +5 | 21.9 | +3 |
| 10–30 | 21.1 | 0 | | |
| | 21.0 | −1 | | |
| | 22.1 | +4 | 21.4 | +1 |
| 10–31 | 21.7 | +2 | | |
| | 21.4 | +1 | | |
| | 22.1 | +4 | 21.7 | +2 |
| 11–1 | 23.0 | +8 | | |
| | 21.3 | 0 | 22.2 | +5 |

TABLE 3—*Concluded*

| DATE, 1922 | TOTAL BASAL CALORIES | VARIATION EACH PERIOD FROM GRAND AVERAGE OF 21.2 CALORIES | DAILY AVERAGE | VARIATION OF DAILY AVERAGE FROM GRAND AVERAGE OF 21.2 CALORIES |
|---|---|---|---|---|
| | | *per cent* | *calories* | *per cent* |
| 11–3 | 20.9 | −1 | | |
| | 21.1 | 0 | | |
| | 22.0 | +4 | 21.3 | 0 |
| 11–6 | 21.3 | 0 | | |
| | 20.0 | −6 | | |
| | 21.1 | 0 | 20.8 | −2 |
| 11–7 | 21.1 | 0 | | |
| | 20.0 | −6 | | |
| | 21.4 | +1 | 20.8 | −2 |
| 11–8 | 20.2 | −5 | | |
| | 20.7 | −2 | | |
| | 19.8 | −7 | 20.2 | −5 |
| 11–9 | 21.3 | 0 | | |
| | 20.9 | −1 | | |
| | 20.7 | −2 | 21.0 | −1 |
| 11–10 | 20.5 | −3 | | |
| | 20.9 | −1 | | |
| | 21.5 | +1 | 21.0 | −1 |
| 11–13 | 18.8 | −11 | | |
| | 19.8 | −7 | 19.3 | −9 |
| 11–14 | 18.8 | −11 | | |
| | 18.1 | −15 | 18.5 | −13 |

For the administration of the adrenalin solution, the vein was exposed and the needle introduced under local anesthesia after the determination of two basal periods; following this procedure, time was allowed for the dog to quiet down to a basal level again, and in the later experiments another control was run before the injection was started in order to be certain that the basal condition was not disturbed by the manipulations incident to the introduction of the needle. The dog always remained motionless and showed no signs of fear or evidence of the slightest discomfort or pain.

The adrenalin solution used was not assayed, but was prepared by taking from 0.10 to 0.50 cc. of Parke, Davis & Company's adrenalin solution 1:1000 and diluting to 50 cc. with 0.8 per cent sodium chlorid

solution. The solution was then introduced into a graduated buret and connected to the intravenous needle by a short, narrow-bored rubber tubing which had been filled with the solution. By means of large gravity bottles a constant air pressure was maintained on the

TABLE 4

*Effect on heat production of the intravenous injection of adrenalin*

Dog F257. Average basal total calories 27.1 (sixty-nine periods)*

| DATE, 1922 | EXPERIMENT | DOSE, FOR EACH KILOGRAM EACH MINUTE | MINUTES | TOTAL DOSE ADRENALIN | AVERAGE BASAL TOTAL CALORIES FOR EXPERIMENTAL DAY | GREATEST INCREASE IN TOTAL CALORIES FROM ADRENALIN | INCREASE OVER BASAL FOR THE DAY | | INCREASE OVER AVERAGE BASAL | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Calories | Per cent | Calories | Per cent |
| 3–20 | III | 0.0011 | 13.0 | 0.25 | 27.1 | 32.7 | 5.6 | +21 | 5.6 | +21 |
| 3–22 | IV | 0.0012 | 6.0 | 0.125 | 26.0 | 33.1 | 7.1 | +27 | 6.0 | +22 |
| 3–22 | V | 0.0023 | 12.8 | 0.50 | 26.0 | 34.3 | 8.3 | +32 | 7.2 | +27 |
| 4–20 | XI | 0.0011 | 12.8 | 0.25 | 27.6 | 36.1 | 8.5 | +31 | 9.0 | +33 |
| 4–22 | XIII | 0.0011 | 6.6 | 0.13 | 26.2 | 33.1 | 6.9 | +26 | 6.0 | +22 |
| 5–8 | XIV | 0.0006 | 12.8 | 0.13 | 25.5 | 33.9 | 8.4 | +33 | 6.8 | +25 |
| 5–9 | XV | 0.0006 | 6.3 | 0.06 | 26.6 | 32.0 | 5.4 | +20 | 4.9 | +18 |

* Average variation of each basal period from 27.1 calories = 1.3 calories or 4.8 per cent.

TABLE 5

*Effect on heat production of the intravenous injection of adrenalin*

Dog F754. Average basal total calories 21.2 (forty-five periods)*

| DATE, 1922 | EXPERIMENT | DOSE, FOR EACH KILOGRAM EACH MINUTE | MINUTES | TOTAL DOSE ADRENALIN | AVERAGE BASAL TOTAL CALORIES FOR EXPERIMENTAL DAY | GREATEST INCREASE IN TOTAL CALORIES FROM ADRENALIN | INCREASE OVER BASAL FOR THE DAY | | INCREASE OVER AVERAGE BASAL | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Calories | Per cent | Calories | Per cent |
| 10–31 | XXV | 0.0006 | 6.5 | 0.05 | 21.7 | 23.8 | 2.1 | +10 | 2.6 | +12 |
| 11–7 | XXIX | 0.0012 | 6.6 | 0.10 | 20.8 | 23.7 | 2.9 | +14 | 2.5 | +12 |
| 11–9 | XXXI | 0.0010 | 12.0 | 0.16 | 21.0 | 24.8 | 3.8 | +18 | 3.6 | +17 |
| 11–10 | XXXII | 0.0012 | 13.1 | 0.20 | 21.0 | 26.7 | 5.7 | +27 | 5.5 | +26 |

* Average variation of each basal period from 21.2 calories = 0.8 calories or 3.7 per cent.

solution in the buret, and its rate of flow was adjusted as desired by the buret tap. The number of cubic centimeters running from the buret each minute was noted by an observer, and if any adjustment in the rate of flow was necessary it was readily accomplished by the

appropriate manipulation of the buret tap. As a result the rate of the injection of the adrenalin was maintained at the desired velocity within a neglible error for the required length of time.

TABLE 6

*Effect on heat production of the intravenous injection of adrenalin*

Dog F513. Average basal total calories 22.1 (forty-eight periods)*

| DATE, 1922 | EXPERIMENT | DOSE, FOR EACH KILOGRAM EACH MINUTE | MINUTES | TOTAL DOSE ADRENALIN | AVERAGE BASAL TOTAL CALORIES FOR EXPERIMENTAL DAY | GREATEST INCREASE IN TOTAL CALORIES FROM ADRENALIN | INCREASE OVER BASAL FOR THE DAY | | INCREASE OVER AVERAGE BASAL | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Calories | Per cent | Calories | Per cent |
| 10–19 | XLIII | 0.0006 | 6.5 | 0.05 | 23.1 | 27.3 | 4.2 | +18 | 5.2 | +24 |
| 10–25 | XLVI | 0.0006 | 6.8 | 0.05 | 23.0 | 26.9 | 3.9 | +17 | 4.8 | +22 |
| Dog F5. Average basal total calories 32.4 (fourteen periods)† | | | | | | | | | | |
| 1–17 | LVI | 0.0025 | 13.0 | 0.50 | 29.8 | 41.2 | 11.4 | +38 | 8.8 | +27 |

* Average variation of each period from 22.1 calories = 0.7 calories or 3.2 per cent.

† Average variation of each period from 32.4 calories = 1.9 calories or 5.9 per cent.

TABLE 7

*Effect on the blood sugar of dog F625 from the intravenous injection of adrenalin at the rate of 0.0016 mgm. for each kilogram each minute for thirteen minutes, or a total of 0.2 mgm.*

| MINUTES BEFORE AND AFTER INJECTION | BLOOD SUGAR | |
|---|---|---|
| | Benedict | Folin |
| Twenty, before | 0.12 | 0.12 |
| Two, before | 0.12 | 0.12 |
| One, after | 0.18 | 0.18 |
| Sixteen, after | 0.17 | 0.17 |
| Forty-six, after | 0.16 | 0.15 |
| Seventy-six, after | 0.13 | 0.14 |

PROTOCOLS. *Explanation of charts.* The pertinent details of all the experiments are recorded here. Instead of presenting the large mass of data obtained in the form of tables, we are reporting the results graphically in charts 1 to 7. The upper curve represents the total calories each hour, the second curve the respiratory quotient, the third curve the pulse rate, the fourth curve the respiration rate, and the bottom curve the ventilation rate each minute. On the line marked *T* is placed the number of minutes during which the intravenous injection lasted, and the arrow indicates the period during which the adrenalin was administered: the injection and the metabolism period were always started

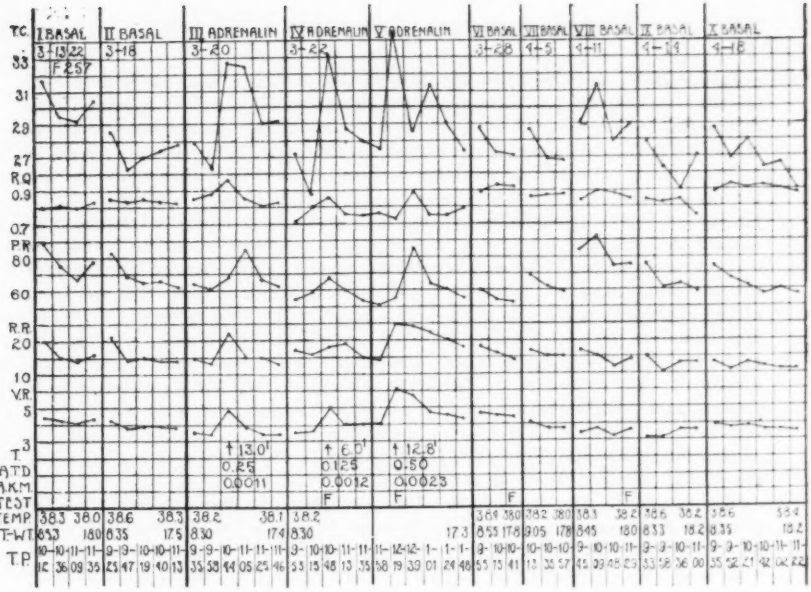



Chart 1

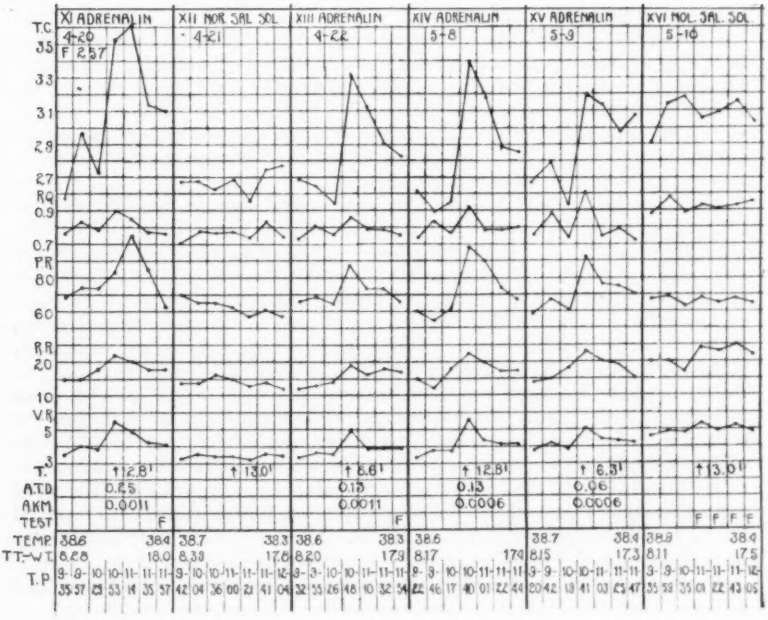



Chart 2

simultaneously, and the latter was almost always fifteen minutes in duration. On the line marked *A.T.D.* is given the total dose of adrenalin administered, expressed as milligrams, which is the same as the number of cubic centimeters of the 1:1000 solution of Park, Davis & Company actually used. The dose of adrenalin for each kilogram each minute is given in the line marked *A.K.M.* On the line marked *Test* is indicated whether or not the period was satisfactory by placing an *F* whenever the period was only fairly satisfactory; when not thus marked the period is to be considered as satisfactory both from a review of the observer's notes and from a study of the kymographic records. On the line marked *Temp.* is placed the rectal temperature (Centigrade) of the dog, obtained at the beginning and end of the experiment. On the line marked *T.T.* – *Wt.* is placed first the time (hour and minute) at which the dog was placed on the table, and the preliminary rest period begun; following this is the weight of the dog for that day in kilograms. On the double line marked *T.P.* is given the hour (above) and minute (below) at which the period charted in that column was begun.

Experiments I to XVI inclusive (charts 1 and 2), were made on a healthy adult mongrel female dog (F257) of unknown age.

*Experiment I. March 13, 1922.* The normals are rather high (30.6). After the second period a hypodermic needle was introduced into the vein, left there a few minutes and then withdrawn. The periods following this do not show elevation of the metabolism as a result of the procedure (29.2, 30.4). Although all periods were satisfactory, according to the observer's notes, this experiment was not included in the normal average because it was the first experiment run on this dog, and in nearly all instances the first experiment on any dog was much higher than succeeding experiments.

*Experiment II. March 18th.* The two normal periods (27.4) were lower than in the preceding experiment. After the second period the needle was inserted into the vein and left in during the first thirteen minutes of the next period, exactly as if an injection were being made. The following periods show no elevation of metabolism as a result of the presence of the needle or the gentle manipulation connected with its removal (27.0, 27.4, 27.8). All periods were satisfactory, according to the observer's notes.

*Experiment III. March 20th.* The two normal periods were satisfactory (27.1). Following the second period the needle was introduced into the vein. Shortly thereafter the injection of a total of 0.25 mgm. adrenalin diluted with 49.75 cc. of sodium chlorid solution was started simultaneously with the beginning of the respiration period. It required thirteen minutes to introduce the adrenalin solution, and as the dog weighed 17.4 kilograms, the rate of injection was 0.0011 mgm. adrenalin for each kilogram each minute. A distinct rise in the metabolism was observed for the period coincident with the injection. The rise was from an average basal of 27.1 calories to a level of 32.7 calories, or an increase of 21 per cent. The next period was started eight minutes after the ending of the injection, and showed, for the following fifteen minutes, a metabolism practically the same as the preceding (32.4 calories, or a 20 per cent increase above the basal). In the next two observations, beginning at twenty-eight and forty-nine minutes after the ending of the injection, the metabolism had returned nearly to normal (29.0 and 29.1 calories). All the periods were satisfactory according to the observer's notes and the pneumographic records.

*Experiment IV. March 22nd.* The normal periods were slightly lower (26.0 calories) than the average normal. Following this a total of 0.125 mgm. of

adrenalin (25 cc. of a solution containing 0.25 mgm. adrenalin in 49.75 cc. of sodium chlorid solution), was injected during the first six minutes of the third period, or at a rate of 0.0012 mgm. for each kilogram each minute. There was a distinct elevation of metabolism amounting to 27 per cent above the day's basal, or 22 per cent above the average basal. The following period starting nineteen minutes after the end of the injection showed a marked return toward normal, and the two subsequent periods were still lower and belong within the normal range of variation. All tests were satisfactory, both according to the observer's notes and the pneumographic records, except the period of injection which was marked as fairly satisfactory because of several slight movements of the right front leg.

*Experiment V* was a direct continuation of experiment IV, and a second adrenalin injection was made one hour and thirty-one minutes after the first. The dose was larger, being a total of 0.50 mgm. adrenalin during the first twelve and eight-tenths minutes of the metabolism period, or at the rate of 0.0023 mgm. adrenalin for each kilogram each minute, accompanied by an increase in metabolism of 27 per cent above the average basal. The sharp drop of the metabolism in the following period with a subsequent rise is somewhat unusual. All periods were satisfactory, except that irregular and shallow respiration during the injection period necessitated the recording of this period as only fairly satisfactory; there were no movements noted, as shown by the graphic record.

*Experiments VI and VII.* March 28th and April 25th respectively the experiments were normal controls.

*Experiments VIII to X.* April 11th, 14th and 18th, respectively, the experiments were run as a second series to determine with certainty the fact that the manipulation incident to the injection was not the cause of the previous rises found after injecting adrenalin. Of the fourteen periods of these three experiments the observers noted that all were satisfactory, except the last period of the eighth experiment during which there were several movements; the metabolism, however was not materially increased. The second period of this experiment, which was by far the highest, was perfectly satisfactory so far as could be judged by the observer; no visible movements occurred.

*Experiment XI. April 20th.* The second of the two normal periods (25.8 and 29.7 calories) was, for some unknown reason, slightly elevated; the average of these two (27.8 calories), however, agrees with the third normal run after the needle had been introduced into the vein (27.3 calories). A total of 0.25 mgm. adrenalin in 49.75 cc. of sodium chlorid solution was then injected at the rate of 0.0011 mgm. of adrenalin for each kilogram each minute for twelve and eight-tenths minutes. The metabolism for this period of injection was 28 per cent above the average of the three normals (27.6). The following period, starting eight minutes after the end of the injection, was even slightly higher, 31 per cent above normal. In the following two periods the metabolism (31.4 and 31.0 calories) materially dropped, but did not reach the normal level fifty-one minutes after the injection. All the periods were satisfactory, except the last one which was recorded as fairly satisfactory, owing to slight movements and restlessness exhibited by the dog.

*Experiment XII. April 21st.* This experiment was identical to experiment XI except that 50 cc. of sodium chlorid, without adrenalin were injected. As may be seen from the curves, the metabolism was in no way affected. All periods were perfectly satisfactory.

*Experiments XIII to XV.* In these three experiments on April 22nd, May 8th and 9th, respectively, the two normals before the introduction of the needle and the normal taken subsequently were satisfactory. All the periods after the injection of adrenalin in each experiment were satisfactory, except the last period of experiment XIII, which was considered fairly satisfactory. In our opinion this series met all the exacting requirements necessary for accurate determinations. In experiment XIII there was an increase in the metabolism of 26 per cent above the day's basal, or 22 per cent above the average basal for a total dose of adrenalin of 0.13 mgm. in six and six-tenths minutes, or at the rate of 0.0011 mgm. for each kilogram each minute. In experiment XIV there was an increase of 33 per cent above the day's basal, or 25 per cent above the average basal for a similar total dose of adrenalin of 0.13 mgm. injected in twice the time (twelve and eight-tenths minutes), or at the rate of 0.0006 mgm. for each kilogram each minute. Although the total dose was the same in experiments XIII and XIV, a slightly greater reaction was obtained by the slower rate of injection. In experiment XV, the third of this series, the total dose was halved (0.06 mgm. adrenalin) and it was injected at the same rate as in experiment XIV, but for half the length of time (six and three-tenths minutes). This minute dose of 0.0006 mgm. for each kilogram each minute for only six and three-tenths minutes caused an elevation in the metabolism of 20 per cent above the day's basal, or 18 per cent above the average basal.

*Experiment XVI. May 10th.* This experiment was run as a control with the injection of sodium chlorid solution containing no adrenalin. This was the third consecutive morning of experiments run all forenoon, and probably as a result the normals obtained on this day were abnormally high. As the normal periods were satisfactory, so far as observer's notes or pneumographic records are concerned, while the subsequent periods were only fairly satisfactory, and as there was no elevation of the metabolism as a result of the injection of sodium chlorid solution, the experiment indicates that the intravenous injection of 50 cc. of sodium chlorid solution does not cause an elevation of the metabolism.

Subsequent to these experiments the dog (F257) was utilized for feeding experiments of various kinds up to July 28, 1922. During this time the following normal controls were obtained: May 23rd, the average of five satisfactory normal determinations was 25.2 calories, and June 15th, the average of four satisfactory determinations was 25.7 calories. Controls one hour after the introduction and removal of a stomach tube were run as follows: July 5th the average of four determinations was 27.7 calories, July 10th the average of six satisfactory determinations was 28.8, July 25th the average of five satisfactory determinations was 25.6 calories. The controls, after the introduction of the stomach tube, are not quite as low or so consistent, as those shown in the adrenalin series. The explanation probably is due to two factors; first, that sometimes more than one hour was necessary, after the use of the stomach tube, before the basal level of metabolism was reached; and second, that the six determinations required the dog to lie quietly in one position for from three and one-half to four hours, resulting occasionally in a slight elevation of one or two calories toward the end of the experimental period. In spite of all this there was a remarkable consistency in the values for the normal periods; the average of sixty-nine normals between March 18th (omitting March 13th), and July 25th, was 27.1 calories, and the greatest variation of any single normal period from this average was +17 per cent, and the average deviation from 27.1 of a single period, regardless of sign, was 1.3 calories (4.8 per cent). The adrenalin experiments are summarized in table 4.

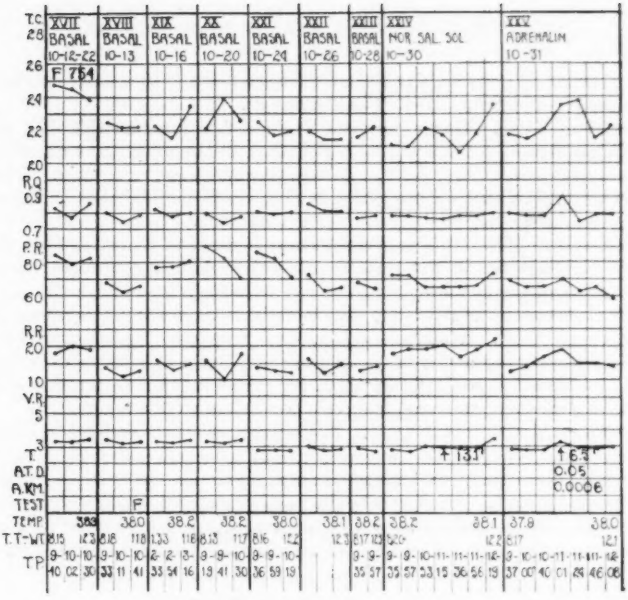



Chart 3

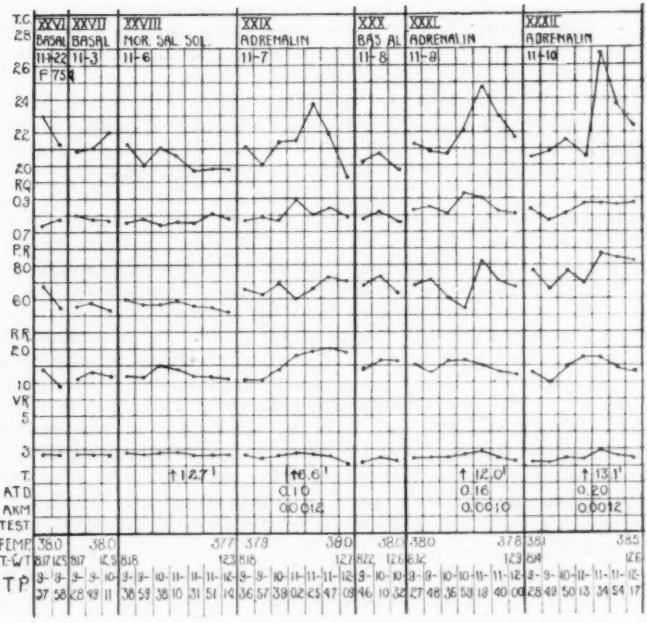



Chart 4

Experiments XVII to XXXII inclusive (charts 3 and 4) were made on a healthy adult, female bull terrier, F754, of unknown age.

*Experiment XVII.* This experiment was the first done on this dog, and as usual the periods were considerably higher than in the subsequent experiments.

*Experiments XVIII to XXIII.* These experiments were a series of basals; in experiments XIX and XX one period in each was rather high, but on the whole, the agreement from day to day was very satisfactory.

*Experiment XXIV.* This experiment was a control, 50 cc. sodium chlorid solution were injected in the same manner as though it contained adrenalin. The elevation of the metabolism in the last period two hours after the injection cannot be interpreted as similar to the immediate rise produced by adrenalin.

*Experiment XXV.* A definite and typical calorigenic reaction was shown, amounting to +12 per cent over the average basal, and to +10 per cent over the day's basal from a dose of 0.0006 mgm. for each kilogram each minute for six and one-half minutes; the total dose only amounted to 0.05 mgm.

*Experiments XXVI and XXVII.* These were normal basal experiments.

*Experiment XXVIII.* This experiment was a control injection using 50 cc. sodium chlorid solution from which there was no increase in heat production.

*Experiment XXIX.* A definite effect was demonstrated, amounting to +12 per cent over the average basal and to +14 per cent over the day's basal from a dose of 0.0012 mgm. for each kilogram each minute for six and six-tenths minutes, or twice the dose of experiment XXV; the total dose was 0.10 mgm.

*Experiment XXX.* This was a normal basal experiment.

*Experiment XXXI.* A greater calorigenic effect was obtained than in experiment XXIX, amounting to +17 per cent over the average basal, and to +18 per cent over the day's basal. Although the dose for each kilogram each minute was slightly smaller (0.0010 mgm.), the total dose was greater, 0.16 mgm., because the injection was continued for twelve minutes instead of six and six-tenths minutes.

*Experiment XXXII.* This was the final experiment of the series, and the dose for each kilogram each minute, 0.0012 mgm., was continued for thirteen and one-tenth minutes, making a total dose of 0.20 mgm. The calorigenic effect caused a rise in metabolism of 26 per cent over the average basal, and of 27 per cent over the day's basal.

The experiments on this dog (F754) were most satisfactory, as the normal basals were satisfyingly constant from day to day as may be seen in table 3. The experiments lasted more than a month, during which time there was no appreciable change in weight; there was, however, a slight and progressive decrease in the heat production, from 6 per cent above the average at the beginning of the month to 13 per cent below at the end of the study. The two control experiments, experiments XXIV and XXVIII, with sodium chlorid solution showed no increase in heat production. With progressively increasing doses, experiments XXV, XXIX, XXXI and XXXII, as summarized in table 5, showed a nearly parallel increase in the calorigenic reaction. An increase in reaction is caused both by increasing the dose for each kilogram each minute, keeping the duration of administration the same, and by increasing the duration of administration and keeping the dose for each kilogram each minute constant. Within the limits of time and dose used, the height of the reaction corresponds most closely to the total dose administered.

Experiments XXXIII to LI inclusive (charts 5 and 6) were made on a healthy adult mongrel male dog, F513, of unknown age.

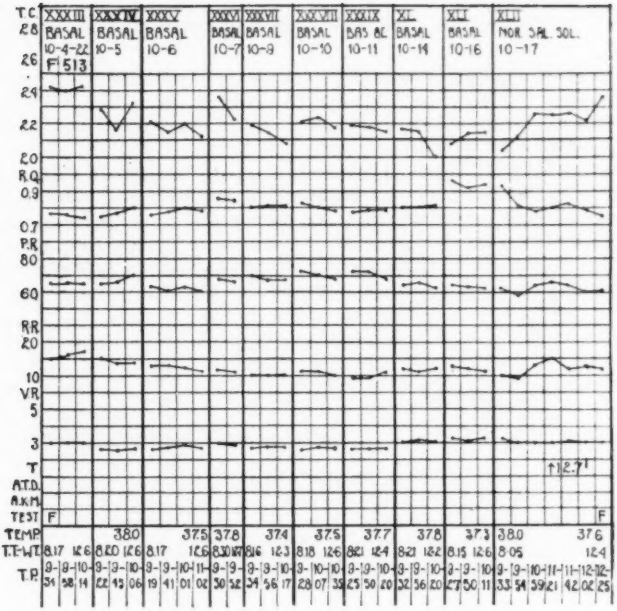



Chart 5

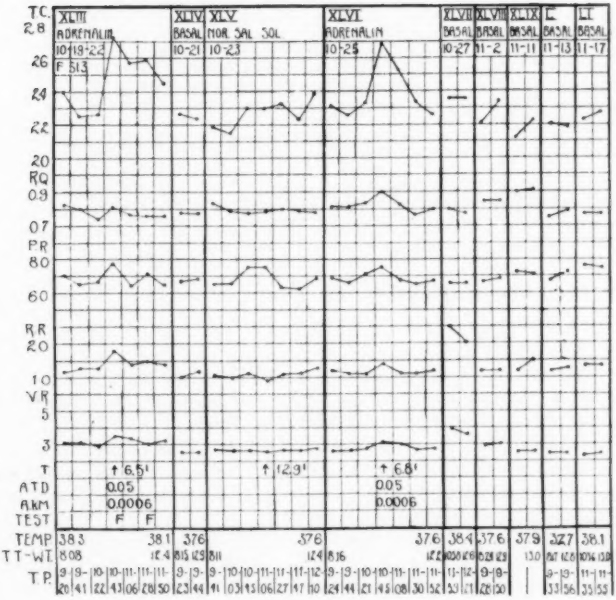



Chart 6

*Experiments XXXIII to XLI.* These experiments were a series of normal basal determinations which, with the exception of experiment XXXIII, are quite satisfactory although an occasional period, without any recognizable cause, is further from the average than we desire.

*Experiment XLII.* This experiment was a control, using 50 cc. sodium chlorid solution. The first three periods were normal basals and showed an undesirable variation, although the succeeding three periods following the starting of the sodium chlorid injection were practically the same as the last basal period; the final period showed a slight increase in metabolism, but the entire curve in no way resembled that obtained when adrenalin was used.

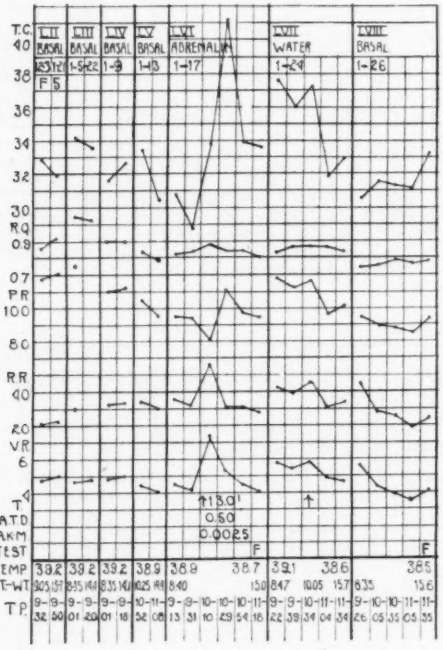



Chart 7

*Experiment XLIII.* A very marked and definite reaction was obtained from the injection of 0.0006 mgm. of adrenalin for each kilogram each minute for six and one-half minutes, making a total dose of 0.05 mgm.

*Experiment XLIV.* This was a satisfactory normal basal experiment.

*Experiment XLV.* This was a normal control using sodium chlorid solution, and it was more satisfactory than experiment XLII, although not quite as satisfactory as similar ones obtained on dog F257.

*Experiment XLVI.* A very marked and definite reaction was obtained on this experiment from the small dose of adrenalin of 0.0006 mgm. for each kilogram each minute for six and eight-tenths minutes, making a total dose of 0.05 mgm.

*Experiments XLVII to LI.* These experiments were final series of normal basal determinations and were satisfactory.

Of the basal experiments on dog F513, the daily average never varied more than 5 per cent from the grand average, except on one day when there was a 7 per cent increase. The two experiments with sodium chlorid solution were not quite as satisfactory as those on dog F754, yet in neither was there an increase in any way comparable to that obtained after the injection of very small doses of adrenalin. The effect of the administration of adrenalin in doses of 0.0006 mgm. for each kilogram each minute for six and one-half and six and eight-tenths minutes, totaling 0.05 mgm., was to cause an increase in the metabolism over the average for the day of 18 and 17 per cent respectively, and of 24 and 22 per cent over the average basal heat production. The magnitude of the calorigenic action in comparison with the variations of the normal basal periods can be best seen from the curves.

Experiments LII to LVIII inclusive (chart 7) were carried out on dog F5, an adult female bull terrier in excellent condition.

*Experiments LII to LV.* These experiments were normal basals and show wider fluctuations than were obtained in our other dogs.

*Experiment LVI.* This experiment shows the effect of a large dose of adrenalin of 0.0025 mgm. for each kilogram each minute for thirteen minutes totaling 0.50 mgm. The reaction obtained was very marked, an increase of 38 per cent over the average basal for the day and of 27 per cent over the average basal heat production.

*Experiment LVII.* This experiment was unsatisfactory; for some unknown reason the three normal periods were unusually high. Subsequent to these 200 cc. of water at 40°C. were given by stomach tube and the two succeeding periods were within the usual range of the normal for this animal.

*Experiment LVIII.* This was a satisfactory normal basal experiment.

On dog F5 only one adrenalin experiment was made. The reaction was very great, amounting to an increase of 27 per cent over the average basal from the administration of adrenalin at the rate of 0.0025 mgm. for each kilogram each minute for thirteen minutes, or a total dose of 0.50 mgm. As may be seen from the curves, there was a greater variation in the basal periods made on this dog during the present series of experiments than was desirable. The cause of these variations is not known, but possibly may be due to incomplete training, as later she became a most excellent subject for determining the effect of other substances on the basal metabolism.

THE CALORIGENIC ACTION OF ADRENALIN. The increases in pulse rate, respiration rate and respiratory volume in the experiments here reported quite closely parallel the increase in the metabolic rate, and are confirmatory evidence of the latter observation. The data seem to leave no question of the increase in heat production following the injection of adrenalin in doses within the accepted powers of the adrenal glands to secrete. The observed increases are much greater than the total technical and physiologic errors inherent in the measurement of the heat production, and the minimal reaction is greater than the largest variation of any single normal period. There is, however, legitimate ground for discussing the cause of this increase

in heat production and especially for excluding muscular movements, as being the source of the extra heat. Visible muscular movements as the cause were excluded by two methods; close observation of the animal during the experimental period with an accurate recording by the observer of all movements, and permanent kymographic records obtained by pneumographs around the upper and lower parts of the body. In all the experiments, except for those indicated, the dog lay quiet, and there was no evidence of increased tonicity or rigidity of the muscles, such as occurs, for example, when a soldier is standing at "attention." All of the experiments were performed without anesthesia; those reported by us last year were performed under complete urethane anesthesia. The experiments under urethane confirm those performed without anesthesia, and show that adrenalin injected when the dog is completely relaxed from anesthesia causes increased metabolism. Experiments on human subjects likewise confirm the conception that the calorigenic effect of adrenalin is not due to muscular movements, because these subjects neither subjectively nor objectively altered the tonus of their muscles. An increase of 20 to 30 per cent of the total heat production of all the tissues excludes the probability that the observed increase was due to a localized effect in some special organ. If such were the case the magnitude of the increase, if limited to one or two organs, would amount to several hundred per cent for that organ. Similarly, the total heat increase cannot be explained quantitatively by any probable assumption of the increased work of the heart or respiratory muscles.

The *respiratory quotient* is almost invariably elevated after the injection of adrenalin, and usually closely parallels the heat production curve. This elevation indicates that the increased heat production is probably, in the main, dependent on an acceleration of a phase of carbohydrate combustion; this is confirmed by the observation of a quite synchronous rise in the blood sugar concentration; in fact, the mobilization of glucose by adrenalin chlorid is a well-known phenomenon and we have demonstrated a slight increase from doses similar to those which produce a calorigenic action (tables 1, 2 and 7).

The mechanism of the increased heat production must be considered as possibly in part due to increase of carbohydrate metabolites, on account of the similarity of the metabolic rate curve following the injection of adrenalin to that found by Lusk and others from a carbohydrate plethora. However, we have previously presented evidence (tables 1, 2 and 7) that the calorigenic action of adrenalin is too great to be accounted for entirely by the slight increase in blood sugar concentration produced by the small doses of adrenalin used in these experiments. It seems more probable, therefore, that the increase

in the blood sugar either is a compensatory mechanism produced by the action of adrenalin as an aid to the increased heat production, or is the consequential result of the reaction.[3]

We believe that our evidence proves that adrenalin in amounts within the probable capacity of the adrenal glands to secrete, accelerates the rate of heat production in the intact animal body, and therefore permits the tentative generalization that one function of the adrenal glands is to furnish a secretion which accelerates the velocity of an as yet unknown chemical reaction, or reactions, in the direction accompanied by the liberation of heat.[4]

## SUMMARY

The object of this paper is to present, as briefly as possible, evidence that adrenalin, when injected intravenously into dogs in doses

[3] Boothby and Wilder at the meeting of the Society for Experimental Investigation, May 1923, presented a series of curves which are published in Medical Clinics of North America, July 1923, showing that insulin does not itself possess a calorigenic action. They showed, however, an increase in the metabolism as the blood sugar falls to what for a given individual is the critical level for the onset of the symptoms of hypoglycemia. At this point the shape of the metabolism curve and the other phenomena observed are so similar to that after administration of adrenalin that the possibility of a spontaneous discharge of adrenalin was evident. They point out that the increase in metabolism at this point seems to be secondary to the low level of the blood sugar or rather to the mechanism that alters the direction of the drop in the blood sugar curve when the latter reaches its critical level. Wilder, Boothby, Barborka, Kitchen and Adams (Jour. Metabolic Research, Dec. 1922, Vol. II, p. 722) described one of these experiments in detail and state that herein we may be dealing with an automatic protective mechanism against hypoglycemia.

It is interesting to note that the calorigenic action of adrenalin as described in this paper is so definite as to be useful in directing attention to a possible protective body mechanism hitherto unsuspected. Cannon, McIver and Bliss (Boston Medical and Surgical Journal, July 26, 1923) following the suggestion of Boothby and Wilder have presented additional evidence supporting this view. Cannon, McIver and Bliss even suggest that this mechanism is concerned with the maintenance of the physiological percentage of blood sugar and is of great importance to the normal status of the body. The three experiments of Stewart and Rogoff (Am. Jour. Physiol., July 1923) in which they found no definite effect of any moment from insulin upon the epinephrin output were performed under different conditions. In their experiments the animals were anesthetized and exhibited a condition of marked hyperglycemia before the injection of insulin and the lowest blood sugar recorded is 143 milligrams for each 100 cc.; therefore their present experiments cannot be considered as invalidating the evidence obtained by the indirect methods of Boothby and Wilder or of Cannon, McIver and Bliss.

[4] Investigations directed toward determining the influence of adrenalin on the Meyerhof glycogen lactic acid reaction would be interesting and might possibly throw more definite light upon the mechanism. The evidence available at present seems consistent with the idea that this reaction might be influenced by adrenalin.

that seem probably within the power of the adrenal glands to secrete, does actually increase the rate of heat production, probably producing this increase by a general and quite parallel increase in the heat production of all or nearly all types of cells. The increased metabolism is accompanied by an elevation of the respiratory quotient, and by a slight increase in the blood sugar concentration, as well as certain other secondary manifestations.

## BIBLIOGRAPHY

(1) Anderes and Cloetta: Arch. f. exper. Path. u. Pharm., 1916, lxxix, 301.
(2) Aub: Journ. Amer. Med. Assoc., 1922, lxxix, 95.
(3) Aub: Personal communication of abstract of preliminary report.
(4) Aub, Forman and Bright: This Journal, 1921, lv, 293.
(5) Aub, Bright and Widal: This Journal, 1922, lxi, 300.
(6) Aub, Forman and Bright: This Journal, 1922, lxi, 326.
(7) Aub, Bright and Forman: This Journal, 1922, lxi, 349.
(8) Aub and Taylor: Endocrinol., 1922, vi, 255.
(9) Barcroft and Dixon: Journ. Physiol., 1906, xxxv, 182.
(10) Bernstein and Falta: Verhandl. d. deutsch. Kong. f. inn. Med., 1912, xxix, 536.
(11) Boothby: Journ. Amer. Med. Assoc., 1922, lxxix, 107.
(12) Boothby and Sandiford: This Journal, 1920, li, 200.
(13) Boothby and Sandiford: This Journal, 1921, lv, 293.
(14) Boothby and Sandiford: This Journal, 1922, lix, 463.
(15) Cannon and Rapport: This Journal, 1921, lviii, 308.
(16) Eppinger, Falta and Rudinger: Zeitschr. f. klin. Med., 1908, lxvi, 1.
(17) Evans: Journ. Physiol., 1912, xlv, 213.
(18) Evans: Journ. Physiol., 1914, xlvii, 407.
(19) Evans and Ogawa: Journ. Physiol., 1914, xlvii, 446.
(20) Fuchs and Roth: Zeitschr. f. exper. Path. u. Therap., 1912, x, 187.
(21) Fuchs and Roth: Orvosi hetil., 1912, lvi, 219.
(22) Fuchs and Roth: Zeitschr. f. exper. Path. u. Therap., 1913, xiv, 54.
(23) Fuchs and Roth: Orvosi hetil., 1913, lvii, 619.
(24) Garrey: Journ. Gen. Physiol. 1921, iii, 41.
(25) Goetsch: N. Y. State Med. Journ., 1918, xviii, 259.
(26) Griffith: This Journal, 1923, lxv, 15.
(27) Hari: Biochem. Zeitschr., 1912, xxxviii, 23.
(28) Hutchinson: This Journal, 1922, lxii, 192.
(29) Jackson: Journ. Lab. Clin. Med., 1916, ii, 145.
(30) Jackson: Experimental pharmacology. St. Louis, 1917.
(31) La Franca: Zeitschr. f. exper. Path. u. Therap., 1909, vi, 1.
(32) Lusk and Riche: Arch. Int. Med., 1914, xiii, 673.
(33) Lusk: The science of nutrition. 3rd. ed. Philadelphia, 1917.
(34) Lusk: Proc. XVII. International Congress of Medicine, Sec. 2, London, 1913, 513.
(35) Marine and Baumann: This Journal, 1921, lvii, 135.
(36) Marine and Baumann: Journ. Metabolic Res., 1922, i, 777.
(37) Marine and Baumann: Journ. Metabolic Res., 1922, ii, 1.
(38) Marine and Baumann: This Journal, 1922, lix, 353.

(39) MARINE AND LENHART: This Journal, 1920, liv, 248.
(40) MARTIN AND ARMITSTEAD: This Journal, 1922, lix, 37.
(41) MARTIN AND ARMITSTEAD: This Journal, 1922, lxii, 488.
(42) PATTERSON AND STARLING: Journ. Physiol., 1913, xlvii, 137.
(43) PEABODY, CLOUGH, STURGIS, WEARN AND TOMPKINS: Journ. Amer. Med. Assoc., 1918, lxxi, 1912.
(44) PEABODY, WEARN AND TOMPKINS: Med. Clin. N. Amer., 1918, ii, 507.
(45) PEABODY, STURGIS, TOMPKINS AND WEARN: Amer. Journ. Med. Sci., 1921, clxi, 508.
(46) PLUMMER AND BOOTHBY: This Journal, 1921, lv, 295.
(47) RUBNER: Die Gesetze des Energieverbrauchs bei der Ernährung. Leipzig, 1902.
(48) SANDIFORD: Clinical metabolism. Papers from the Mayo Foundation and the Medical School of the University of Minnesota, Philadelphia, 1915–1920, i, 527–529.
(49) SANDIFORD: This Journal, 1920, li, 407.
(50) SCOTT: Journ. Exper. Med., 1922, xxxvi, 199.
(51) STEWART AND ROGOFF: Journ. Pharm. Exper. Therap., 1917, x, 1.
(52) TOMPKINS, STURGIS AND WEARN: Arch. Int. Med., 1919, xxiv, 269.
(53) VON NOORDEN: Die Zuckerkrankheit und ihre Behandlung. Berlin, 1912.
(54) WELECKI: Bull. de l'Acad. d. sci. de Cracovie, 1909, 119.
(55) WILENKO: Lwow. tygodn. lek., 1912, vii, 343.
(56) WILENKO: Biochem. Zeitschr., 1912, xlii, 44.

# PHASE REVERSAL IN EMULSIONS AND PROTOPLASM

WILLIAM SEIFRIZ

*From the Osborn Botanical Laboratory, Yale University*

Received for publication May 19, 1923

There is no conception concerning the fundamental physical nature of protoplasm which has been more widely accepted than that protoplasm is an emulsion, not only in its coarse, microscopic structure but in its ultramicroscopic, colloidal structure as well. Associated with this idea is that of phase reversal in the living emulsion. Many interesting hypotheses are based on the belief that protoplasm is an emulsion which may exist in either the "sol" or "gel"[1] state, and that in the change from one state to another there takes place a reversal of phases.

An emulsion is a system in which one liquid is dispersed in the form of small droplets in another liquid in which it is immiscible. Phase reversal is that phenomenon which involves an interchange in the relative positions of the two liquid phases which make up an emulsion; that is, that liquid which is at first the dispersed phase of the liquid-liquid system becomes the continuous phase after reversal has taken place, and so also does the originally continuous phase become the discontinuous or dispersed phase. The commonly cited example of phase reversal is that which takes place when milk is transformed into butter.

Numerous vital phenomena, such as viscosity changes, changes in the configuration of the cell during mitosis, pseudopod formation, etc., have been interpreted as solution-gelation processes without necessarily implying that a reversal of phases takes place. Other similar theories which purport to explain the changes which take place in the permeability of protoplasm, are based on an actual interchange in the relative positions of the phases of the supposed, ultramicroscopic, living emulsion. Such hypotheses are those of Herlant and Clowes.

Herlant (9) was of the opinion that mitosis in certain eggs is accompanied by a reversal of the two phases, lipoid and protein, in the plasma membrane. During the early stages of mitosis the membrane is believed to be a protein-in-lipoid emulsion. Such a membrane would be impermeable, or poorly permeable, to water-soluble substances, but

[1] The word "gel" applied to the firm condition of an emulsion is a very obvious misuse of the term. Gels (and jellies) are fundamentally quite different systems from firm emulsions.

readily permeable to lipoid-soluble substances. Herlant bases this speculation on experimental results which show that during the prophases of mitosis the permeability of the cell membrane is most disturbed by water-soluble substances such as salts and strong bases, while during the anaphases of cell division it is the fat-soluble substances such as ammonia and saponin which disturb the diosmotic properties of the membrane the most. Somewhat similar is the hypothesis of Clowes. It is with this hypothesis that we are here primarily concerned.

THE HYPOTHESIS OF CLOWES. Clowes' (6) hypothesis is based on an analogy drawn between the behavior of protoplasm and that of oil and water emulsions. Clowes discovered that if in an emulsion of oil and water, in which soap is the emulsifier, the hydroxide of the monovalent cation Na is in excess, the emulsion is one of the oil-in-water type, while if the salt of a bivalent cation (e.g., $CaCl_2$) is in excess, the system is of the water-in-oil type. The emulsions used by Clowes were made by shaking equal volumes of olive oil and of aqueous solutions of NaOH and $CaCl_2$ of varying concentrations. The soap used as the emulsifier was either added directly or, in most cases, obtained through emulsification, by the NaOH, of the oleic acid in the olive oil. When Na is in excess, sodium oleate is the emulsifier formed, and when Ca is in excess, the emulsifier is calcium oleate. Clowes observed that when there is an excess of NaOH in the emulsion, and sodium oleate is therefore the emulsifier, the type of the emulsion is oil-in-water, and when $CaCl_2$ is in excess, and the emulsifier is therefore calcium oleate, the emulsion is of the water-in-oil type. Clowes saw in this behavior of oil emulsions a close analogy to the reaction of protoplasm to mono- and bivalent cations.

The extensive work which has been done on the antagonism of salts and on the effects of salts on protoplasmic permeability is so well known to biologists that any detailed discussion of it is unnecessary here. Suffice it to say that, chiefly as a result of the work of Osterhout, it is now generally accepted that all monovalent cations (except H) increase permeability of the living cell while all bivalent cations decrease permeability (12). It might be mentioned that not all biologists have fully accepted this conclusion of Osterhout. Fitting (7) believes that certain monovalent cations (he worked primarily with KCl) lower permeability. In some work of my own (13) I have preferred not to attempt to interpret in terms of permeability the changes in osmotic pressure occasioned by Na, on the one hand, and Sr on the other, but feel convinced that there has, in the case of Na, been at least no increase, while in the case of Sr there was every indication of an increase in permeability, the reverse of what Osterhout maintains.

An emulsion of oil dispersed in water is a system which is readily permeable to water-soluble substances, and an emulsion of water dispersed in oil is a system which is poorly or not at all permeable to water-soluble substances. Since Na (in the form of the hydroxide and in the emulsions with which Clowes worked) produces an emulsion of the former type, and (in the opinion of Osterhout) increases protoplasmic permeability, and since Ca produces an emulsion of the latter type and decreases the permeability of protoplasm, Clowes conceives of the living membrane as a fine emulsion which is in a state of equilibrium at or near the reversal point. In the presence of Na the emulsion at the surface of protoplasm would tend more toward the oil-in-water type, and in the presence of Ca it would approach the water-in-oil condition, never being fully of the one or the other type while the cell is living. Clowes looks upon living protoplasm in contact with water as a system which is, within the protoplasmic mass, a dispersion of proteins, lipoids, etc., in water and, at its surface, a system of the reverse type of structure in which water is dispersed in a continuous fatty or lipoid phase. The dispersion at the surface is not, however, complete, but rather a system in which the fatty continuous phase is permeated by water channels, i.e., it is at the reversal point. This sponge or honey-comb structure of the surface layer of protoplasm Clowes considers as capable of being modified by calcium salts, anesthetics, etc., in such a way that a system is formed in which the water communications are decreased or completely cut off. Salts of monovalent cations, on the other hand, would tend to produce a system in which the water channels are more extensive.

Clowes' hypothesis is based on three assumptions: first, that protoplasm is, in its colloidal nature, an emulsion; second, that this emulsion is capable of phase reversal; and third, that the emulsifier active in protoplasm is either soap or a substance which behaves as does soap when in the presence of certain mono- and bivalent cations. Without intending to assert that the first assumption is untrue, it is nevertheless well to point out that we are totally ignorant of the actual physical nature of the ultramicroscopic particles of the living colloid. This being the case, any convenient hypothesis can be postulated. One cannot, therefore, deny the possibility of protoplasm being a colloidal emulsion, that is, a system in which liquid, ultramicroscopic particles, in a high state of dispersion, are suspended in a liquid medium. The actual physical nature of the colloidal particles of the living system we cannot yet hope to determine,but after all, as Hatschek (8) points out, the distinction between liquid and solid becomes rather vague with particles approaching ultramicroscopic dimensions.

Regardless of the physical nature of the living colloidal particles, there is no evidence upon which to base the assumption that a reversal of phases takes place in protoplasm. Protoplasm is a lyophilic colloid. Phase reversal was formerly thought to be the process by means of which lyophilic colloidal sols, "emulsoid" sols, become gels (jellies). The researches of Bachmann (1) and Zsigmondy (14), which have been substantiated by a different method of investigation by McBain (10), have shown that in gelatin (Bachmann) and soap (McBain) no reversal of phases takes place in the transformation of the gelatin sol and the soap sol into their gels.

It is not my desire here to question further the emulsion theory of protoplasmic structure. I do, however, wish to emphasize the fact that the ideas that protoplasm is, in its *colloidal* nature, an emulsion, and that this living emulsion is capable of phase reversal, are pure assumptions. But we shall, with Clowes and others, tentatively assume that protoplasm is a colloidal emulsion in which a reversal of phases is a physical possibility provided that the other prerequisite conditions, such as the type of emulsifier, are favorable. It is with this last assumption, namely, the type of emulsifier, that we are here primarily concerned. That Clowes' hypothesis is highly interesting and that it opens up a new line of speculation on the mechanism of permeability changes, goes without saying. The question is, how far is the theory applicable?

ACKNOWLEDGMENT. I had the pleasure of discussing the hypothesis of Clowes with Mr. Hatschek of London. I objected to Clowes' analogy on the grounds of the first two of the three assumptions named above. Mr. Hatschek then called my attention to the third assumption, namely, that if some emulsifier other than soap is used, the oil-in-water emulsions will not reverse no matter how much salt of a bivalent cation is added. This was demonstrated by a simple test-tube experiment in which olive oil was emulsified in a weak aqueous solution of saponin. An excess of $BaCl_2$ failed to cause a reversal. It was this demonstration and subsequent discussion which ultimately led to the research work here published. I wish not only to acknowledge the fact that the original suggestion on which this article is based comes from Mr. Hatschek, but I also desire to express my indebtedness to him for turning over to me the notes on his preliminary experiments.

I wish also to make known my indebtedness to Prof. H. S. Uhler of the Department of Physics of Yale University, who so kindly placed a small laboratory and all necessary physical instruments at my disposal.

METHOD. All of the emulsions worked upon were prepared by adding 25 cc. of oil to 25 cc. of an aqueous solution of the emulsifier. The mixture was put into a 150 cc. flask and vigorously shaken intermittently. This method of preparing an emulsion is that of Briggs (4), and con-

sists in giving the mixture of oil and aqueous solution ten violent shakes by hand, then allowing the emulsion to rest for half a minute, when it is again shaken. This procedure is repeated nine times.

The oil used was the commercial "Pompeian" olive oil. The emulsifiers were all aqueous solutions. Where the organic stabilizer is insoluble in water, it was dissolved in some suitable solvent, such as alcohol, and a colloidal dispersion made by pouring a small quantity of the alcoholic solution into water. The emulsifiers were the following: the two soaps, sodium oleate and sodium stearate; gelatose; gum arabic; the glucosides: saponin, senegin and smilacin; the proteins, albumin from egg, albumin from blood, casein and gliadin; the lipoids, cholesterin and lecithin, the alkaloid, cephalin; and an extract from plant tissue. To these solutions was added a small crystal of thymol except in those cases where the solution itself is aseptic.

The electrolytes used in reversing or in attempting to reverse the emulsions were NaOH, NaCl, $Na_2SO_4$, $BaCl_2$, $CaCl_2$ and $Ba(OH)_2$. The emulsions were thoroughly shaken after each addition of salt or alkali. While no attempt was made to obtain in every case precise quantitative measurements, yet the reversal point in all instances was determined with reasonable accuracy. To ascertain the amount of electrolyte necessary to cause reversal was not the main objective, but merely to determine whether or not a reversal is at all possible with certain emulsifiers and certain ions.

We shall first concern ourselves with the type of emulsifier. Two electrolytes only will, at the beginning, be considered in reference to reversibility. The question whether other electrolytes have the same influence as do NaOH and $BaCl_2$, will be taken up after the question of the type of emulsifier is disposed of. A further problem which developed during the earlier experiments, namely, the possible influence of the degree of acidity of the emulsions on their type and reversibility, is to be discussed in a subsequent publication.

Three methods were followed in determining the type of emulsion: 1, electrical conductivity; 2, color; 3, microscopical examination.

The electrical conductivity method is based on the principle that since water is the continuous phase in an oil-in-water emulsion, the emulsion will conduct a current of electricity, while an emulsion of the reverse type, since oil is the continuous phase, will not conduct an electric current. This method was first proposed by Clayton (5, p. 114) and, has more recently been used with success by Bhatnagar (2). The type of emulsion prevailing is ascertained by the reading of an ammeter. The reversal point can be determined with considerable precision. The method also has the advantage over the color method of telling in a remarkable way what is happening in the emulsion. A higher or lower

reading, the rising or falling of the needle, and particularly the fluctuations of the ammeter needle, are all indicators of what is taking place in the emulsion.

An Arrhenius or Ostwald conductivity cell can be conveniently used for making the conductivity measurements, but equally satisfactory and much less expensive is a simple apparatus consisting of two electrodes of platinum foil 1 cm. wide and 10 cm. long supported by two glass rods 2 cm. apart. With this apparatus the emulsions to be tested are poured into small glass vials, measuring 3 cm. in diameter and 9 cm. high with a volume of 68 cc. The platinum electrodes are allowed to project into the vials any convenient distance, but the same distance in every case—in these experiments to a depth of 5 cm. in the emulsion. The current used in this work was a 52 volt direct current. The ammeter employed was a milliammeter reading to one milliampere, with a range of 150 milliamperes. For readings in excess of 150 milliamperes a shunt was inserted in the circuit which raised the value of the milliammeter scale tenfold.

The readings of the oil-in-water type of emulsions varied from 3 milliamperes in the case of the soap (sodium sterate) emulsions, to, with one exception, 100 milliamperes in the case of the gelatose emulsions. In those soap (sodium oleate) emulsions in which the aqueous phase is a 1M NaOH solution, the current reached the high value of 420 milliamperes. Higher amperage is obtained in all emulsions which have been repeatedly reversed by alternate additions of mono- and bivalent electrolytes.

The color method for determining the type of emulsion consists in adding a stain which is soluble in but one of the two phases. For this purpose the oil soluble dye Sudan III may be used. When the emulsion is of the oil-in-water type the color is a pale pink. When the emulsion is of the water-in-oil type its color is a brilliant red, since the oil is then the continuous phase.

The microscopic method of determining the type of emulsion consists simply in making a microscopic examination of a sample of the emulsion. This method is not as convenient as the other two but should always be employed to determine the precise state of an emulsion, especially when it is incompletely reversed. It sometimes happens that the electrical conductivity points clearly to an emulsion of the oil-in-water type, while the color indicates equally clearly that the system is of the reverse type. Only a microscopical examination reveals the true state of affairs.

EXPERIMENTAL. *Sodium oleate.* The first emulsion studied was the same as that with which Clowes worked, namely, an emulsion in which the emulsifier is sodium oleate. The sodium oleate is formed as a result

of saponification of the oleic acid in the olive oil by the NaOH in the aqueous phase of the emulsion. The emulsion is made by shaking 25 cc. of olive oil with the same volume of the aqueous phase. Molar and M/10 NaOH were used. The emulsion is quickly formed and is of the oil-in-water type. The dispersed oil droplets measure from mere specks of less than $\frac{1}{2}\mu$, which are in active Brownian movement, to globules $50\mu$ in diameter. The viscosity of the emulsion is 5.7 as compared with a value of 1 for water. The current which will pass through such an emulsion at 52 volts is 420 milliamperes in the case of lM NaOH, and 100 milliamperes when M/10 NaOH is the aqueous phase. On the addition of $BaCl_2$ the current falls. One would expect a rise in amperage when the concentration of electrolytes in the continuous aqueous phase is increased, and this is what takes place in those oil-in-water emulsions which do not reverse. The fall in current in a soap emulsion indicates a reduction in the volume of the electrolytic phase which is available as a conductor for the current. This reduction, as evidenced by the fall in amperage, is an indication that the emulsion is reversing. Complete reversal of the M NaOH emulsion takes place when 1.2 cc. of M $BaCl_2$ has been added. The M/10 NaOH emulsion is reversed with 0.3 cc. of M $BaCl_2$, or 3 cc. of M/10 $BaCl_2$. The milliammeter reads 0.00 after complete reversal has taken place. A very slight reading, of some 3 milliamperes, may be obtained when the color of the emulsion plainly indicates that there has been reversal. This is due to an excess of aqueous phase which has not been caught in the water-in-oil emulsion. If the emulsion is allowed to stand for a short time, less than a minute, when the excess of free water settles in a small layer at the bottom, no reading of the ammeter is to be had.

*Sodium stearate.* This emulsion was prepared by adding the soap directly to the aqueous phase. A M/1,000 concentration of sodium stearate ($NaC_{18}H_{35}O_2$) was used. A good emulsion is formed in which the oil particles measure from 1 to 30 $\mu$. The emulsion creams very slowly. Its viscosity is 100 as compared with 1 for water. The emulsion is an oil-in-water one, and is readily reversible with $BaCl_2$. Its electrical resistance is high, the current passing through it, at 52 volts, being but 3 milliamperes. It is very sensitive to electrolytes, requiring but 0.2 cc. of a M/1,000 concentration of $BaCl_2$, and but 2.0 cc. of a M/10,000 concentration of $BaCl_2$ to reverse it. The water-in-oil emulsion thus obtained by reversal can be readily reversed to its original type by adding NaOH. It was possible, by alternate additions of $BaCl_2$ and NaOH, to reverse a sodium stearate emulsion nine times. High concentrations of the two electrolytes were used to avoid too great a dilution of the emulsion. One drop, 0.033 cc., of M $BaCl_2$ is sufficient to reverse the original emulsion.

*Gelatose.* This emulsifier was prepared by boiling 10 grams of gelatin in 100 cc. of water for ten hours in a reflux condenser. The hot sol was filtered through a tight plug of cotton wool. The solution thus obtained is non-gelatinizable. A so-called WH Gold Label brand of gelatin (supplied by Paul Putmann, Inc., New York) was used. Gelatose and olive oil produce an oil-in-water emulsion with a viscosity 30 times that of water. The amperage of the gelatose emulsion varies from 92 to 116 milliamperes, averaging about 100. Reversal cannot be produced in this emulsion by as strong a concentration as 10 cc. of M $BaCl_2$. As the volume of the aqueous phase in 50 cc. of emulsion was, in all cases, 25 cc., the final concentration of $BaCl_2$ in the system was $1000 \times \frac{10}{35} = 286$ millimoles. This concentration, at which neither reversal nor "breaking" of the emulsion takes place, is more than 3,500 times that necessary to reverse the soap (sodium stearate) emulsions mentioned above, and about 15,000 times as great as the concentration which Bhatnagar (3) finds necessary to cause reversal in the emulsions with which he worked.

*Saponin, senegin and smilacin.* These saponin solutions were made from Merck preparations. The saponin, senegin and smilacin were dissolved in water at concentrations of 1 in 500. Each of these glucosides produce oil-in-water emulsions which cannot be reversed by $BaCl_2$.

*Gum arabic.* Ten grams of a fine, white, powdered gum arabic were dispersed in 100 cc. of water. With this solution as an emulsifier the resulting emulsion is of the oil-in-water type. The amperage of the original emulsion is, with a 52 volt current, 78. On the addition of 0.1 cc. of M $BaCl_2$ the current rises to 81 milliamperes; and finally to 250 milliamperes when 10 cc. of M $BaCl_2$ have been added. The oil-in-water emulsion with a gum arabic emulsifier is, therefore, irreversible with $BaCl_2$.

*Albumin.* Two kinds of albumin were used, albumin from blood and albumin from egg, both Merck products. Albumin readily forms an oil-in-water emulsion in which the oil particles measure from 1 to 50$\mu$. The emulsion creams slowly, a barely noticeable aqueous layer being formed at the bottom in 5 minutes. The viscosity of an emulsion made from 1 per cent blood albumin is 9; from $\frac{1}{2}$ per cent egg albumin it is 4.6, as compared with 1 for water. The amperage at 52 volts is, in 1 per cent blood albumin, 91, and in $\frac{1}{2}$ per cent egg albumin, 39 milliamperes. An emulsion with either of the two albumins as the emulsifier cannot be reverted by 10 cc. of M $BaCl_2$ in 50 cc. of emulsion.

*Casein.* Casein well shaken in water and allowed to stand for an hour gives a fine colloidal dispersion which readily emulsifies olive oil. The emulsion formed is a water-in-oil one, and suddenly becomes stable

during the sixth or seventh interval of shaking, i.e., after 60 or 70 shakes. The dispersed water particles are from less than $\frac{1}{2}$ to 30 $\mu$ in diameter, and exhibit no Brownian movement. The emulsion is very thick, the viscosity being too high to conveniently measure with a capillary viscosimeter. No current will pass through the original emulsion at 52 volts. The emulsion reverts readily with NaOH. One drop (0.033 cc.) of M NaOH will bring the emulsion to the reversal point. Two drops will cause complete reversal into an oil-in-water emulsion. If to this emulsion one drop of M $BaCl_2$ is now added, the emulsion immediately reverts to its original type, i.e., to a water-in-oil one, the ammeter reading being again 0.00. By alternately adding 1 or 2 drops of M NaOH and M $BaCl_2$, it was possible to reverse an emulsion with a casein emulsifier eight times.

*Gliadin.* This alcohol soluble protein, which comes from wheat gluten, was kindly given me by Dr. Thomas B. Osborne of the Connecticut Agricultural Experiment Station. The solution received was a 5 per cent concentration of gliadin in 70 per cent alcohol. Two cubic centimeters of this were dispersed in 98 cc. of water. The colloidal solution thus obtained was used as the emulsifying agent. The resulting emulsion is a water-in-oil one. The emulsion forms very quickly, usually within the first ten shakes. The color of the original emulsion is red (the oil phase being stained with Sudan III), indicating clearly that oil is the continuous phase. If the emulsion is not completely formed a slight (3 milliampere) reading of the ammeter may be obtained. Ordinarily, however, the ammeter registers zero.

A water-in-oil emulsion with 0.1 per cent gliadin as the aqueous phase, is readily reversed by 0.3 to 0.5 cc. of M/10 NaOH into an oil-in-water emulsion, with a milliamperage of 6 or 7. Two-tenths cubic centimeter of M/10 $BaCl_2$ will again reverse the emulsion into its original form, with a zero reading of the ammeter; and 0.5 cc. of M/10 NaOH will throw it back again to an oil-in-water emulsion with a milliamperage of 10. By keeping the emulsion near the reversal point it can be reversed again and again by very small quantities of salt and base. When thus kept near the reversal point, 0.07 to 0.1 cc. of M/10 $BaCl_2$ will produce complete reversion into a water-in-oil system with which no reading of the milliameter can be obtained, and 0.15 to 0.3 cc. of M/10 NaOH will cause reversal in the opposite direction with a milliamperage increasing with each reversal, i.e., with increasing concentration of electrolytes in the aqueous phase. At the eleventh reversal the milliamperage was 60.

*Lecithin.* Lecithin is a lyophilic "lipoid" readily dispersed in water. The lecithin used came from Merck. One gram was dispersed in 100 cc. of water. This sol makes a very good emulsifying agent. The

emulsion formed is of the oil-in-water type, the oil particles of which vary from 1 to 8 or 10$\mu$ (relatively few larger) in size, the smaller ones being in active Brownian movement. The milliamperage of the emulsion, with a 1 per cent lecithin aqueous phase, is 30. Ten cubic centimeters of M $BaCl_2$ raise the amperage to 320 milliamperes, which means that the emulsion is still of the original oil-in-water type. The oil emulsion with lecithin emulsifier is, therefore, irreversible with $BaCl_2$.

*Cholesterin.* Cholesterin is generally included among the "lipoids". It is more true to the character of fatty substances than is lecithin, being lyophobic. An aqueous colloidal dispersion of cholesterin was made by first dissolving 2 grams in 100 cc. of absolute alcohol boiled in a reflux condenser, and dispersing 1 cc. of this 2 per cent alcoholic solution in 99 cc. of water, and finally filtering. This 0.02 per cent aqueous dispersion of cholesterin emulsifies olive oil very quickly into a water-in-oil emulsion of high viscosity.

It is remarkable how precisely these cholesterin emulsions will emulsify at exactly the same point in the process of shaking when the emulsions are made from the same lot of material. Of three cholesterin emulsions made up at the same time two emulsified on the 55th shake and one on the 58th. The pauses between every tenth shake were, in these three emulsions, $\frac{1}{2}$ minute in the first case, 1 minute in the second case, and 5 minutes in the third case. The length of the pause, if above a certain minimum, is apparently of little or no influence.

No current, at 52 volts, passes through the original water-in-oil cholesterin emulsion. The emulsion is readily reversible with NaOH. One drop (0.033 cc.) of M NaOH, or 0.3 cc. of M/10 NaOH, is sufficient to reverse the emulsion. $BaCl_2$ will reverse the oil-in-water emulsion obtained with NaOH, back into the original type, and NaOH will again cause reversal into an oil-in-water emulsion.

*Cephalin.* The alkaloid cephalin was used in a concentration of 1:500 as an emulsifier. With this solution as the aqueous phase a good water-in-oil emulsion is obtained. Complete reversal results on the addition 1.0 to 1.5 cc. of M/10 NaOH. This oil-in-water emulsion can be reversed back to the original form by 0.3 cc. of M/10 $BaCl_2$. One cubic centimeter of the M/10 NaOH will again reverse the emulsion, and 2.0 cc. of M/10 $BaCl_2$ will again bring it back. As the emulsion is reversed again and again higher concentrations of the salt and the base are necessary to cause complete reversal. Finally, the emulsion "breaks," that is, separates into two layers, of oil and water respectively.

*Plant extract.* A quantity of fresh spinach was run several times through a coarse and then a fine mincer. The pulp thus obtained was subjected to high pressure. A large quantity of plant juice, consisting of protoplasm and cell sap, is pressed out. This plant extract will

remain in a state of colloidal suspension for two or three days. It then precipitates. Such an extract from spinach, diluted to one-half, forms, with olive oil, a good oil-in-water emulsion, the oil particles of which are from 1 to 50$\mu$ in diameter, averaging some 5 or 10$\mu$. The emulsion has a viscosity of 10.7 as compared with 1 for water. The current obtained with 52 volts is high, being 235 milliamperes. Small quantities of M/10 to 10 cc. of M BaCl will not cause this emulsion to reverse.

THE INFLUENCE OF OTHER IONS ON REVERSIBILITY. We have seen how highly speculative a theory may be when formulated on the behavior of one type of emulsion. Equally misleading would it be to look no further than the effect of a single group of ions.

In considering the reversibility and irreversibility of the emulsions, the influence of only two electrolytes $BaCl_2$ and NaOH has so far been considered, the former being used to reverse the emulsions of the oil-in-water type and the latter to reverse those of the water-in-oil type. The hydroxide of barium and the chloride of calcium were also tried upon the oil-in-water systems, and two salts of sodium were tried upon the water-in-oil emulsions. In every case $CaCl_2$ had the same effect on the emulsions as did $BaCl_2$, reversing the reversible ones and having no effect on the irreversible ones. This was also true for $Ba(OH)_2$, with one exception, to be considered shortly.

The fact that the hydroxide of barium will reverse the sodium oleate emulsion in which the aqueous phase is the hydroxide of sodium, is interesting because of its bearing on the relative importance of cations and anions on reversibility. One-tenth cubic centimeter of saturated $Ba(OH)_2$ (which titrated 0.194 M) has no apparent effect on 50 cc. of sodium oleate emulsion in which M/10 NaOH is the dispersion medium. Two-tenths cubic centimeter of the $Ba(OH)_2$ will reverse $\frac{1}{4}$ of the total volume of the emulsion: 0.4 cc. will reverse slightly more than half: and 0.7 cc. will reverse the entire 50 cc. of the emulsion into one of the water-in-oil type. A high concentration of NaOH is now necessary to bring the emulsion back into the oil-in-water type. One cubic centimeter of 10 M NaOH will reverse the emulsion into its original form. The concentration of NaOH in the system is still not too high to prevent a third reversal of part of the total volume of the emulsion. One cubic centimeter of saturated $Ba(OH)_2$ will reverse $\frac{3}{5}$ of the already twice reversed emulsion. That portion of the emulsion which has been reversed for the third time is a good water-in-oil emulsion (microscopically examined), of high viscosity.

Barium hydroxide will not only reverse all the emulsions which $BaCl_2$ will, but will also (partially) reverse one upon which $BaCl_2$ has no apparent effect, namely, the emulsion in which gelatose is the emulsifier. A saturated aqueous solution of $Ba(OH)_2$ (0.194 M) will reverse slightly

more than half of the volume of the gelatose emulsion. That portion of the total volume which has been reversed into a water-in-oil system does not separate out into a separate layer distinct from the remaining oil-in-water emulsion, but instead, the two systems are dispersed one within the other. The amperage of the partially reversed emulsion is higher than that of the original, thus indicating that no reversal has taken place. The color of the emulsion, however, has become deep red (the oil phase being stained with Sudan III). One would, therefore, with color alone as the criterion, say, without hesitation, that the emulsion has reversed into one of the water-in-oil type. Only a microscopical examination reveals the true state of affairs, namely, that both types of emulsion exist.

The most remarkable fact about the reversal of the emulsion with a gelatose emulsifier is that the hydroxide causing reversal may be either that of a mono- or bivalent cation. NaOH also partially reverses the emulsion just as does $Ba(OH)_2$.

When an emulsion with a gelatose emulsifier is microscopically examined after 6 cc. of M/10 NaOH has been added it is seen to consist of the two types of emulsions, as just described. Further addition of the hydroxide causes no further reversal but, on the contrary, a *return* to the original oil-in-water system throughout the mixture.

Leaving the unusual behavior of the emulsion with a gelatose emulsifier out of consideration, it can be stated that both the chloride and the hydroxide of barium and of calcium have the same effect in reversing oil emulsions.

When salts of sodium are added to water-in-oil emulsions which are reversible with NaOH, *no* reversal takes place. Neither NaCl nor $Na_2SO_4$ will cause any of the emulsions to reverse. It is quite evident, therefore, that it is not the cation Na which is alone responsible in causing reversal. Nor can we say that it is the anion OH which is alone the active ion in reversing water-in-oil systems, otherwise the hydroxide of any cation should do, yet $Ba(OH)_2$ does not reverse water-in-oil systems, but, on the contrary, behaves like $BaCl_2$ in reversing oil-in-water emulsions.

The inability of NaCl to reverse the emulsions which can be reversed with NaOH, is of special interest because of its bearing on the biological significance of Clowes' hypothesis. It is this salt, NaCl, which has been the classical example to illustrate the effect of a monovalent cation on the permeability of protoplasm, and with $CaCl_2$ furnishes the fundamental evidence on which the theory of antagonism is based. If NaCl has no apparent effect on the reversibility of oil emulsions, the hypothesis of Clowes loses much of its weight.

Clowes (6, p. 423) states that while NaCl does not reverse the water-in-oil emulsions it "undoubtedly promotes the formation of emulsions of oil in water." If this is true, the effect is certainly exceedingly slight. When 5 M NaCl is slowly added to a water-in-oil emulsion in which gliadin is the emulsifier, no change is at first to be noted, either in the (red) color or in the electrical conductivity of the original emulsion. Not until 5 to 7 cc. of 5 M NaCl have been added to a water-in-oil emulsion, with gliadin as stabilizer, is a slight reading of the milliammeter to be had. But the emulsion is still red, and therefore primarily of the water-in-oil type. A microscopical examination reveals the presence of a water-in-oil emulsion in which are scattered larger regions of pure water, indicating a tendency toward separation of the two phases. That NaCl tends rather to cause separation of the water and oil than to promote the formation of an oil-in-water emulsion, is indicated further by the behavior of soap emulsions in the presence of NaCl.

It occasionally happens that an emulsion made with soap (0.001 M $NaC_{18}H_{35}O_2$) as the emulsifier, is of the water-in-oil type, instead of the usual oil-in-water form. This is the case when the soap solution has stood for several days. There takes place a change in acidity, in pH value, which seems to be an influencing factor. No matter what the type, the sodium stearate emulsion is very sensitive to electrolytes. When of the usual oil-in-water type (made with a freshly prepared soap solution), a drop or two of M/10 $BaCl_2$ is sufficient to cause complete reversal. If of the oil-in-water type (made from an old soap solution), very little NaOH is also necessary to cause reversal. If NaCl tends to promote the formation of a system of the oil-in-water type one would expect that this influence would be readily detectable in so sensitive an emulsion as is the one with a sodium stearate stabilizer. Such is not the case, however. Two cubic centimeters or 5 cc. of 5 M NaCl does not prevent the formation of a water-in-oil emulsion in which (an old and therefore less alkaline solution) of sodium stearate is the emulsifier, even though the emulsion is very sensitive to NaOH.

$Na_2SO_4$ is as ineffective as NaCl in causing reversal of the emulsions studied.

Discussion. If speculations on the behavior of the protoplasmic membrane are to have any value, it would appear desirable to ascertain if the reversal of phases by electrolytes of mono- and bivalent cations is a phenomenon common to all emulsions.

It was pointed out in the introduction of this article that Clowes' hypothesis rests on three assumptions. For the purpose of discussion the first two of these assumptions were considered as valid. The third assumption has to do with the type of emulsifier. The question which we can put to ourselves is this, Is the emulsifier that is active in proto-

plasm an emulsifier that will permit reversal of phases in the living emulsion (assuming with Clowes that protoplasm is an emulsion) when the salt or hydroxide of a mono- or bivalent cation is added? A brief review of the foregoing experimental data is sufficient to show that an answer to the question at hand is impossible.

That protoplasm contains soaps we know, but their proportion, compared with that of "lipoids" and proteins, is small. It seems unlikely that soap is the emulsifier that is the determining one in the behavior of the hypothetical protoplasmic emulsion. All experiments on permeability tend to emphasize the presence of proteins and "lipoids" in the plasmatic membrane. If the emulsifier active in protoplasm is a "lipoid," then the question arises whether it is like lecithin, in which case reversal could not happen, or like cholesterin in which case reversal could take place. Lecithin is more abundant in plant tissue, and cholesterin in animal tissue. Whether the "lipoid" is free and functions as such is doubtful. Osborne (10) finds that of a maximum content of 30 per cent of "lipoid" in egg yolk very little is free, the greater portion being chemically bound with proteins.

The abundance of proteins in protoplasm makes it highly probable that the predominating emulsifier is a protein. But this does not simplify our problem. If the emulsifier is an albumin then reversal is not possible, while if it is casein or gliadin, or a protein behaving like them, then reversal is possible. Who can say what the actual nature of the substance is which keeps the supposed protoplasmic emulsion in a state of equilibrium?

Clowes' hypothesis suffers not only from the fact that emulsions with certain kinds of emulsifiers will not reverse, but also from the fact that those emulsions which do reverse do so on the addition of the hydroxide but not the chloride of sodium. The fact that NaCl will not reverse any of the emulsions is of importance in its bearing on the behavior of protoplasm, because it is with NaCl that such pronounced changes in protoplasmic permeability have been observed by many biologists.

In view of the extremely slight if any tendency of NaCl to produce the formation of water-in-oil emulsions, and especially, in view of the inability of NaCl to reverse any of the emulsions here reported upon, it must be evident that Clowes' hypothesis can not be considered to cover the mechanism of the changes in protoplasmic permeability by sodium chloride at least.

Clowes has given us some interesting suggestions. If it should be found that protoplasm is, in its colloidal structure, an emulsion, and that this emulsion is maintained by an emulsifier which permits a reversal of the liquid phases making up the system, then and only then can the speculations of Clowes serve as the foundation of a theory of the

mechanism of permeability changes in the protoplasmic membrane. Before we attempt to visualize so complex a vital phenomenon on the basis of so simple a mechanical process as phase reversal in emulsions, it is only the part of wisdom to thoroughly investigate the assumptions on which our analogy rests.

## SUMMARY

1. Olive oil emulsions in which one of the soaps, sodium oleate or sodium stearate, is the emulsifier, are oil-in-water systems which are reversible with $BaCl_2$.

2. Oil emulsions with casein, gliadin, cholesterin, or cephalin as the aqueous phase, form water-in-oil systems which are reversible with NaOH.

3. Oil emulsions in which saponin (senegin, smilacin), gelatose, gum arabic, albumin, lecithin, or plant extract, is the emulsifier, form oil-in-water systems which are not reversible with $BaCl_2$.

4. Since the hypothesis of Clowes, on the mechanism of permeability changes in the plasma membrane, rests on the assumption that the emulsifier in protoplasm is of that type which form an emulsion that is reversible with $BaCl_2$ or NaOH and since certain of the substances here experimented with as emulsifiers, are very abundant in protoplasm but form emulsions which are not reversible with $BaCl_2$ or NaOH, one is forced to conclude that, since we are totally ignorant of the actual nature of the emulsifier in protoplasm, a theory of the mechanism of permeability changes which is based on the behavior of only one type of emulsion in the presence of certain ions, must be regarded as a purely speculative hypothesis which rests on very uncertain evidence.

5. $CaCl_2$ and $Ba(OH)_2$ are both like $BaCl_2$ in their influence on the emulsions investigated, with one exception.

6. NaCl will not reverse any of the emulsions here investigated. The effect of NaCl on the emulsions is to cause "breaking," that is, to cause a separation of the two liquid phases. There is little evidence to support the belief that NaCl tends to promote the formation of oil-in-water emulsions.

7. In view of the striking difference in the reaction of emulsions to NaCl and to NaOH, there seems to be little reason to believe that the behavior of oil emulsions when treated with NaOH serves as a satisfactory basis of an explanation of the mechanism of permeability changes occasioned in the plasma membrane by NaCl.

8. Since both the chloride and the hydroxide of barium and the chloride of calcium will reverse certain oil-in-water emulsions, and since the chloride of sodium will not reverse certain water-in-oil emulsions which

the hydroxide of sodium will reverse, and since the hydroxide of both sodium and barium will (partially) reverse one particular emulsion, one is forced to conclude that valency of the cation is not alone the determining factor in phase reversal. Nor does it seem possible to formulate any general rule regarding the relative potency of cations and anions in causing reversal.

9. The behavior of emulsions varies with and is dependent upon the type of emulsifier.

## BIBLIOGRAPHY

(1) Bachmann: Zeitschr. f. anorg. Chem., 1912, lxxiii, 125.
(2) Bhatnagar: Journ. Chem. Soc., 1920, cxvii, 542.
(3) Bhatnagar: The phys. and chem of colloids, Rep. Faraday Soc.—Phys. Soc. London, 1921, 27.
(4) Briggs: Journ. Phys. Chem., 1920, xxiv, 120.
(5) Clayton: Second report of the B. A. A. S. on colloid chemistry, 2nd ed., 1921, 96.
(6) Clowes: Journ. Phys. Chem., 1916, xx, 407.
(7) Fitting: Jahr. f. wiss. Bot., 1917, lviii, 553.
(8) Hatschek: Second report of the B. A. A. S. on colloid chemistry, 2nd ed., 1921, 16.
(9) Herlant: Arch. d. Biol., 1920, xxx, 517.
(10) Laing and McBain: Trans. Chem. Soc., 1920, cxvii, 1506.
(11) Osborne and Campbell: Journ. Amer. Chem. Soc., 1900, xxii, 413.
(12) Osterhout: Bot. Gaz., 1915, lix, 317.
(13) Seifriz: Bot. Gaz., 1923, lxxvi.
(14) Zsigmondy: Kolloidchemie, Leipzig, 1920.

# THE EFFECT OF GASTRIN AND HISTAMINE ON THE MOVEMENTS OF THE STOMACH AND INTESTINE DURING HUNGER

A. C. IVY AND D. A. VLOEDMAN

*Department of Physiology, Loyola University School of Medicine*

Received for publication June 7, 1923

It is well known that gastrin when injected intramuscularly causes a marked and quantitative secretion of gastric juice, beginning ten to fifteen minutes after injection and lasting from two to three hours. Carlson (1) has shown that acid (0.5 per cent HCl) introduced into the stomach via a gastrostomy causes an inhibition of hunger movements, the contractions reappearing before all the acid has been neutralized or ejected from the stomach and that gastric juice of full normal acidity (0.4 to 0.5 per cent) has a similar effect. Also, when the contractions are inhibited or depressed by chewing palatable food, the contractions reappear before there is cessation of the psychic secretion of gastric juice. It is also known that during a hunger period a continuous secretion of gastric juice containing free acid occurs.

Heidenhain (2) observed thirty years ago that the intravenous injection of tissue extracts causes a temporary augmentation of intestinal peristalsis probably secondary to the asphyxia that occurs as a result of the fall in blood pressure. Henriques and Hallion (3) reported that extracts of intestinal mucosa caused contractions of the intestine. Zuelzer (4) in a series of poorly controlled experiments conceived the hypothesis of a specific gastro-intestinal motor hormone elaborated in the intestinal mucosa during digestion, absorbed into the blood and stored in the spleen, based upon the observation that extracts of the spleen and intestinal mucosa, when injected intravenously or intramuscularly, caused normal peristalsis. These extracts were tried in the clinic with alleged good results in cases of "intestinal stasis." Well-controlled experiments by Dittler and Mohr (5), Schlagenweit (6), Sabatowski (7) and others showed that intramuscular injections of "hormonal" had little or no effect on gastro-intestinal movements, while intravenous injections caused toxic symptoms typical of all tissue extracts. Weiland (8) reported that extracts of the intestinal mucosa or muscularis stimulate the intestine much more than other organ extracts, their action being antagonized by weak solutions of atropine. He

believed that the active principle of the intestinal extract is the cause of the automatic movements of the excised intestine.

Because of the alleged action of intestinal extracts on gastro-intestinal motility and of the known action of gastrin on the secretion of gastric juice, the following questions suggested themselves for solution: *a*, What is the effect of injection of gastrin on the movements of the stomach and intestines? *b*, Is the resulting secretion of gastric juice related to a possible increased motility of the stomach and intestine? and *c*, Are hunger contractions inhibited by the augmented flow of gastric juice?

METHODS. For recording the movements of the stomach the balloon method outlined by Carlson (1) was used. For the intestine a special balloon was devised, by placing a rubber balloon (condom rubber) about the last five inches of a small rubber tube, the portion of the rubber tube within the balloon being perforated every quarter of an inch. The rubber tube within the balloon made possible the insertion of the rubber balloon without kinking so that it would lie evenly in the intestine. Three dogs with gastrostomy, one with a Thiry's fistula of the duodenum and jejunum and one with a Thiry's fistula of the jejunum and ileum were used. Observations were made on one other dog by passing a balloon into the stomach by way of the mouth.

A known and proven active preparation of gastrin, prepared by the Koch method, was used, which had a latent period of action of from eight to ten minutes. Injections were made intramuscularly and in two instances intravenously. The animals were starved for from twenty-four to forty-eight hours.

The animals were trained to lie quietly and to remain so during the injections. Thirty cubic centimeters of air were put in the balloon in the stomach and from 5 to 10 cc. in the balloon in the intestine.

Observations were made on length of rest and hunger periods, height and frequency of contractions in the case of the stomach and in the case of the intestine on the force and frequency of the rhythmic segmentation, the tonus and the occurrence of tonus rhythm.

Pfansteihl's histamine (ergamine acid phosphate) was used. One milligram of this product injected subcutaneously caused a marked flow of gastric juice in a Pavlov pouch dog. The latent period of action was from five to ten minutes. The pouch secreted 16 cc. of strong gastric juice in one hour, which was the maximum amount yielded by this pouch when the dog was given a meal of meat.

RESULTS. *Gastrin. a. Stomach:* Since gastrin is an irritating substance and causes some discomfort when injected intramuscularly we naturally observed inhibition of the hunger contractions. But after several injections, the animals do not seem to be disturbed and the con-

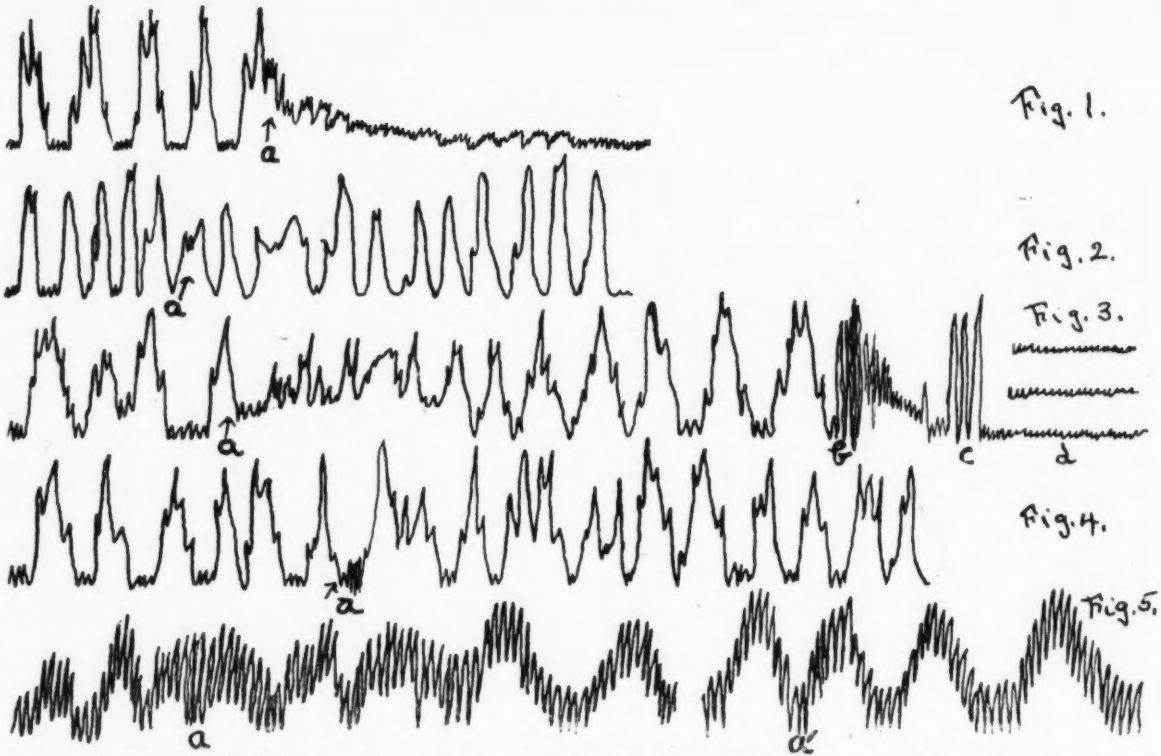



Fig. 1. Shows complete inhibition of hunger contractions following the subcutaneous injection of 2 cc. of gastrin. This is due to reflex inhibition from the site of injection in a dog unaccustomed to the injection. *a*, point of injection.

Fig. 2. Shows only very slight temporary inhibition of contractions in a dog that had been accustomed to the injection of gastrin. In every case the hunger period continued to completion. *a*, point of injection.

Fig. 3. Shows temporary inhibition of contractions following intravenous injection of 0.5 cc. of gastrin, *a*, and complete and prolonged inhibition resulting from the intravenous injection of 1.0 cc. of gastrin. *b*, panting; *c*, vomiting; *d*, complete inhibition.

Fig. 4. Shows no inhibition or alteration of contractions of the stomach following the subcutaneous injection of 1 mgm. of histamine. *a*, point of injection.

Fig. 5. Shows no effect following the subcutaneous injection of 1 mgm. of histamine at *a* and of 2 cc. of gastrin at *a'* on the movements of the intestine. The variations shown are within the normal variations that we have observed in the same an

tractions were either not or only slightly inhibited. The injection had no definite effect on the length of the rest or hunger period, the height rate or type, of the contraction. If the animals had been starved only twenty-four hours, inhibition would sometimes result following injection. This inhibition we believe was a reflex effect from the site of the injection.

One cubic centimeter of gastrin intravenously caused marked and prolonged inhibition of contractions; while 0.5 cc. intravenously caused only a temporary inhibition.

*b. Intestine:* We have failed to observe a single instance in which 2 cc. of gastrin injected subcutaneously has had any effect on the movements of the intestine, the rhythmic segmentations and variations in tone being uninfluenced. Any variations that occurred were within the observed limits of normal variation.

*Histamine.* We studied the action of histamine on the movements of the stomach and intestine during hunger because it is a constituent of extracts of gastric mucosa (9) and because it is known to stimulate gastric secretion when injected subcutaneously (10). In this respect histamine has an action quite similar to that of gastrin.

Quagliariello (11) observed that although histamine causes contractions of the intestine, the uterus is more susceptible to its action. Vauysek (12) reported that histamine causes intense stimulation of the intestine, which action was intensified by the action of calcium.

The observations of these investigators do not have a direct bearing on our work because we have used unanesthetized normal animals and "physiological doses" of histamine, or doses that we know stimulate gastric secretion without having a general systemic toxic effect. Increased motility following toxic doses may be interpreted as asphyxial secondary to the fall in blood pressure. Observations made on intestinal strips we consider questionable, because such experiments are unphysiological.

*a. Stomach:* When doses of histamine (0.5 to 1.0 mgm.) known to stimulate gastric secretion were injected subcutaneously, reflex inhibition being controlled, gastric hunger contractions are not effected. Two milligrams of histamine subcutaneously caused shivering, restlessness and vomiting. In only one instance did we observe any general toxic effect from 1 mgm. of histamine subcutaneously, the symptoms being panting and an abortive vomiting movement. In this case the hunger contractions were only temporarily inhibited.

We know that 1 mgm. of the histamine we used stimulated gastric secretion when injected subcutaneously in a Pavlov pouch dog. These data are given under our methods. The secretion of intestinal juice is also markedly augmented by histamine.

*b. Intestine:* The subcutaneous injection of from 0.5 to 1.0 mgm. of histamine failed to have any effect on the movements of the intestine, both the tonus waves and rhythmic segmentations being unaffected. Any variations that occurred following injections were within the normal limits.

## SUMMARY

Our results warrant the conclusion that gastrin and histamine in "physiological doses" have no effect on the movements of the stomach and intestine during hunger or the interdigestive period. Therefore, the secretion of gastric juice, pancreatic and intestinal juice following the injection of gastrin and histamine is not secondary to increased motility of the stomach and intestine, but to some other as yet unknown factor. Our results also demonstrate that hunger contractions and intestinal movements are not affected by an augmented flow of gastric juice and pancreatic juice that occurs following an injection of gastrin or histamine, which is comparable to the amount of secretion resulting from the ingestion of a test meal of meat. This observation is contrary to the effect of gastric juice introduced into the stomach by tube, in which condition hunger contractions are temporarily inhibited (1), but agrees with Carlson's (1) observation on the effect of psychic secretion of gastric juice on hunger contractions.

## BIBLIOGRAPHY

(1) Carlson: The control of hunger in health and disease. Chicago, 1919.
(2) Heidenhain: Loc. cit. by Carlson, Forscheimer-Billings' System of medicine, Supplement to vol. 1.
(3) Henriques and Hallion: Compt. rend. Soc. biol., 1904, lvi, 322; Ibid., 1911, lxxi, 488.
(4) Zuelzer, Dohrn and Marxer: Berl. Klin. Wochenschr., 1908, xlv, 2065.
(5) Dittler and Mohr: Zeitschr. f. Klin. Med., 1912, lxxv, 275.
(6) Sabatowski: Wien. Klin. Wochenschr., 1912, xxv, 116.
(7) Schlaginweit: Arch. Internat. Pharm., 1913, xxiii, 77.
(8) Weiland: Arch. f. d. gesammt. Physiol., 1912, cxlvii, 171.
(9) Abel and Kuboda: Journ. Pharm. Exper. Therap., 1919, xiii, 243.
(10) Keeton, Luckhardt and Koch: This Journal, 1920, li, 469.
(11) Quagliariello: Zeitschr. Biol., 1914, lxiv, 263.
(12) Vanysek: Biochem. Zeitschr., 1914, lxvii, 221.

# SEASONAL CHANGES IN THE GASES AND SUGAR OF THE BLOOD AND THE NITROGEN DISTRIBUTION IN THE BLOOD AND URINE OF THE ALLIGATOR

ALEITA HOPPING

*From the Department of Physiology, Columbia University, New York City*

Received for publication June 15, 1923

The original purpose in investigating the blood of the alligator was to follow any changes occurring in the blood of a poikilothermic animal, when the temperature of the environment was varied. The carbon dioxide production and oxygen utilization in poikilothermic animals follow closely changes in surrounding temperatures, although there is evidence of regulatory control of metabolism within a range of temperature of from 15° to 20°C. (1). Complex changes occur in the blood of homoiothermic animals under conditions of high environmental temperatures, many of which are directly related to the mechanism of temperature control (2). Changes occurring in the blood following a change of environmental temperature ought to be more directly related to the environmental conditions in poikilothermic than in homoiothermic animals.

In the search for a suitable cold-blooded animal, the American alligator was selected because it offers the advantage of suitable size and the consequent possibility of supplying sufficiently large quantities of blood without serious disturbances due to hemorrhage.

Methods for securing the blood were worked out and preliminary analyses of the gases of the blood were made. It was found that the carbon dioxide contents were markedly lower in January and February, when the work was begun, than in the following March. In some cases there was a difference as great as twenty volumes per cent. In searching for a cause of these variations it occurred to us that they might be due to an annual rhythm, and it became necessary to temporarily discontinue the work as originally planned and to pursue the question of these seasonal variations.

The importance of seasonal changes is obvious in this connection, for it would be impossible to interpret the reaction of the animal to experimental conditions if it is at the same time responding to an unknown and uncontrolled cycle of changes, due not to its immediate environment, but to its individual or inherited history.

Extensive investigations of the nitrogen metabolism of birds have been carried out showing that uric acid is the end product of protein metabolism. Ammonia and lactic acid (3) are combined to form uric acid which also originates from amino acids (4), urea (5), ammonia (6) and from the purin bases (7).

Reptiles are generally classed with birds in their nitrogen metabolism since the excrement of the snake has been found to consist of ammonium urate (8). Among reptiles, however, there is a greater diversity in structure than among birds, and it was thought that investigation of the nitrogen end products in the alligator might add to our knowledge of the biochemistry of this class of animals.

Apparently the blood sugar of the alligator had never been determined previous to this investigation and it was felt that it should be included with the other factors studied.

Discussion of methods. *I. Biological. a. General care of animals:* We thought it advisable to maintain our animals under conditions as nearly uniform as was practical. A wooden zinc-linked tank was built, having dimensions of 5 feet $\times$ 2 feet $\times$ 2 feet. The cover was divided into three sections, the middle section being of glass, and the two end sections of heavy muslin, for ventilation. Small holes were bored through the upper portion of the tank to insure more complete ventilation. The temperature of the water in the tank was controlled by means of a hot water heating system, an electric stove being used as a source of heat. The temperature of the tank was maintained at approximately 26.5°C., a variation of 1 or 2 degrees occurring during sudden changes of weather. The water was changed once a week, and the tank cleaned. The water was heated to the proper temperature before being poured into the tank. The animals were fed twice a week during the spring and early summer, and weekly for the remainder of the period. Raw beef cut in strips was used exclusively. Each time the animals were used for experimental purposes, they were weighed and measured.

Seven alligators were kept in the tank during the fifteen months of the investigation, from January 1922 to March 1923, and at the end of the period they were to all appearances as healthy as at the beginning.

*b. Method of drawing blood.* Blood was drawn from the heart by means of a hypodermic needle with the precautions necessary for blood gas analysis according to the method described by Van Slyke (9). The heart beat is weak and difficult to detect by touch, and so the heart was located by means of a fluoroscopic examination. It was found to be well down in the middle of the body, between the ribs, and unprotected by the sternum. Later it was located in each animal by laying the animal on its back and examining it from the side, when a slight rise

and fall of the skin could be noted. This is essentially the same method as was used by Tiegel (10) in locating the heart of the snake. The horny scales of the epidermal skeleton are arranged in the ventral portion of the trunk in a very regular manner and it is possible by means of the transverse rows to locate the heart at the eleventh and twelfth row from the broad band just above the fore limbs. A needle two inches in length, gauge no. 20, was used. Coagulation was prevented by potassium oxalate.

Saturated oxalate solution was drawn through the needle just before using, and the needle dried by drawing air through it. To obtain the blood sample the needle is inserted through the skin, between the scales, for venous blood, about 0.5 cm. to the right of the midline of the body. When the purpose was to secure arterial blood, the needle was inserted 0.5 cm. to the left of the midline. In all cases, in searching for the heart the needle was directed parallel to the midline, being pushed gently through the tissue below the skin, very slowly, waiting for the detection of the heart beat. When the beating heart is felt at the end of the needle, a "diaphragmatic" expiration is awaited. The needle is then made to follow the heart downward. The needle is held in the final position, and the inspiration that follows pushes the heart upon the needle. By this technic the needle is inserted in the heart to approximately the same depth. Blood samples have been taken at intervals of two weeks throughout the year without the loss of a single animal.

*c. Method of catheterization.* Samples of urine were obtained by catheterizing the cloaca. The catheter was sterilized with 5 per cent carbolic acid, and inserted into the cloaca. The urine was collected in a small graduated cylinder, at times under oil. To prevent infection, the cloaca was washed out with 20 cc. of a saturated solution of boric acid, then washed with 20 cc. of sterilized distilled water.

In the alligator the rectum and ureters open into the cloaca. A sphincter muscle separates the rectum from the cloaca so that the cloaca acts as a bladder. The urine was collected many times, and was always found to be a limpid fluid and never contaminated with fecal matter.

*II. Chemical methods. a. Blood gases.* Estimation of the gases of the blood was carried out by means of the methods described by Van Slyke and Stadie (11). It was found necessary in making the oxygen determinations to add several more drops of ferricyanide than directed by Van Slyke, in order to completely liberate the oxygen of the blood; otherwise the method was followed exactly as described. The oxygen capacity of the blood was determined and the amount of hemoglobin calculated by multiplying by the factor 0.746. By a method for deter-

mining small amounts of iron, described by Brown (13), the hemoglobin was calculated from the iron and compared with that obtained by gas analysis, as a means of control. Instead of using alveolar air for the determination of the carbon dioxide capacity of the blood, samples of blood were exposed to a determined tension of 40 mm. of mercury of carbon dioxide. Ten minutes in a slowly rotating Squibb funnel were allowed for equilibration.

*b. Blood sugar.* The sugar of the blood was determined by the MacLean method (14), as modified by Hastings and Hopping (15).

*c. Nitrogen of the blood.* Total nitrogen of the blood was estimated by the Macro-Kjeldahl method (16). Urea was determined by the method described by Myers (17) and ammonia by the method also described by Myers (17), except that 3 cc. of blood were used instead of 2. In both cases the liberated ammonia was collected in N/50 $H_2SO_4$, and titrated against N/50 NaOH. Urea was determined also by Folin's method on the tungstic acid filtrate (18). Uric acid was determined by Benedict's new method (19), and in a few cases, by the Folin method. Creatine, creatinine and non-protein nitrogen were determined by Folin's method (18), the non-protein nitrogen also by a Macro-Kjeldahl method, titrating with N/50 alkali.

*d. Urinary nitrogen.* Total nitrogen of the urine was determined by the Macro-Kjeldahl method as described for blood. Urea was determined by the method of Van Slyke and Cullen (20). In carrying out the urea determination it was necessary to make the urine faintly acid to litmus before addition of urease. Ammonia was determined by the Steele modification of Folin's method (21). The ammonia content of the urine was so high that the equivalent of 0.2 cc. of urine was used for estimation of ammonia. Urease was used in tablets made by Squibb, also in powder form as made by the Arlington Chemical Company. Uric acid was determined by Benedict's latest method (21).

We wish to express here our appreciation of the help given us by Professor Benedict in carrying out this investigation.

*e. Chlorides.* The chlorides in the blood were determined by the method of Austin and Van Slyke (23). In the urine, the chlorides were determined by the method of Volhard-Harvey (24).

DISCUSSION OF RESULTS: *1. Health of animals.* The value of information gained by experiments carried out over a period of more than a year, upon animals kept under laboratory conditions and subjected to treatment that might be regarded as injurious, must depend largely upon evidence that the animals have not been rendered abnormal. The usual criteria by which such questions are decided are increase in weight and continued growth of the animal. Measurements of length were made over a period of six months and these showed an average increase

in length of 6 cm. for the period, or 1 cm. a month (table 1). According to measurements collected at the New York Zoölogical Garden, under the most favorable conditions, this animal was found to grow about a foot a year (25). Our figures are only 40 per cent of this number but, as mentioned above, the feeding of our animals was cut down considerably and was less than that as described at the Zoölogical Garden. Records of weight were kept and figure 1 shows that for each animal there was a gradual and steady increase. There is an exception to this statement in the earlier portion of the curve, in the case of animals 1 and 2. This was due to the difficulty experienced in finding suitable food. The flattening of the curve which is noted at the middle of the summer was due to the reduction in the amount of food at this time which was mentioned above. This reduction was necessary since the animals were becoming so large that they threatened to outgrow our facilities for handling them.

TABLE 1

*Increase in length from June to December, 1922*

| NUMBER OF ANIMAL | INITIAL LENGTH | FINAL LENGTH | DIFFERENCE |
|---|---|---|---|
| | *cm.* | *cm.* | *cm.* |
| 1 | 98.4 | 108.0 | 9.6 |
| 2 | 88.1 | 93.5 | 5.4 |
| 3 | 65.4 | 72.4 | 7.0 |
| 4 | 101.0 | 105.0 | 4.0 |
| 5 | 108.0 | 115.0 | 7.0 |
| 6 | 86.0 | 90.0 | 4.0 |
| 7 | 96.6 | 103.0 | 6.4 |
| Mean | | | 6.2 |

Since the animals gained in weight and increased in length it may be presumed that they were not seriously injured by the experimental conditions to which they were subjected.

*2. General character of the blood.* The blood drawn from the left side of the heart is bright red and that from the right side dark, purplish red in color. The general appearance of the blood is quite similar to that of the dog or man. The mean volume percentage of corpuscles is 14.5. Under the microscope the red corpuscle is a pale yellow, oval cell, with a large nucleus. The average sizes of the erythrocytes are given below.

| | |
|---|---|
| Length | 24.89–26.52$\mu$ |
| Width | 12.24–16.32$\mu$ |
| Thickness at center | 4.08$\mu$ |

The corpuscles, thus, are larger than human corpuscles. The average number of red corpuscles in the seven animals studied is 850,000 per cu.mm. of blood. The extreme range in different individuals was 616,000 to 1,500,000. The observations from which these averages are obtained are given in table 2.

A leucocyte count was made on alligator 5 in the latter part of March, 1922, and was found to be 8,000 per cu.mm. of blood. The red count at that time was 760,000 per cu.mm. Toison's solution was used in making these blood counts since the leucocytes are stained by the methyl-violet in this solution. A year later a blood count was made on the same animal by Miss Morrison of the Vanderbilt Clinic. The count then was 760,000 for the red blood cells, but 446,000 for the

TABLE 2

*Corpuscular volume and red cell count*

| NUMBER OF ANIMAL | CORPUSCULAR VOLUME | | RED CELL COUNT | |
|---|---|---|---|---|
| | Date, 1922 | Volume, per cent | Date, 1922 | Number per cu. mm. |
| 1 | 5–11 | 17.8 | 3–28 | 680,000 |
| 2 | 4–11 | 13.7 | 3–21 | 732,000 |
| 2 | 5–2 | 13.1 | 4–11 | 616,000 |
| 4 | 2–23 | 16.0 | | |
| 5 | 2–23 | 15.8 | 3–23 | 730,000 |
| 5 | 4–6 | 14.0 | 3–21 (1923) | 760,000 |
| 6 | 3–2 | 11.4 | 3–13 | 881,000 |
| 6 | 5–9 | 11.4 | 3–30 | 800,000 |
| 6 | | | 4–18 | 745,000 |
| 7 | 4–18 | 16.1 | 3–13 | 1,480,000 |
| 7 | 5–9 | 15.5 | 4–4 | 1,130,000 |
| 7 | | | 4–25 | 1,050,000 |

leucocytes. Leucocyte counts were not made during the year, but a great increase in the number of leucocytes was observed when the red cells were counted. The ratio of leucocytes to red cells, on these determinations, is as 1 to 95, at the first observation, and about 1 to 2 on the second. In man, the values in normal blood are 5,000 to 9,000 per cu.mm. of blood, and about 5,500,000 erythrocytes, or a ratio of 1 to 600 or 1,100. Anything above 10,000 may be regarded as pathological, and in spleno-myelogenous leukemia the leucocyte count may rise to 500,000. It is an interesting fact that the animal that gave this high leucocyte count was in an apparently healthy condition. The study of this remarkable leucocytosis is being continued.

*3. Blood gases.* The oxygen contents and capacities of the blood and the carbon dioxide contents and capacities were determined throughout the year on six animals.

In following the oxygen content of the blood of an individual animal through a series of observations it is necessary to show that previous hemorrhage has presumably not modified the succeeding determinations.

Whipple (26) has shown that the hemoglobin is replaced in the dog following a hemorrhage of 50 per cent after about three weeks. No work upon the blood volume of the alligator has been reported but

TABLE 3

| DATE | NUMBER OF ANIMAL | OXYGEN CAPACITY PER 100 CC. BLOOD | | IRON PER 100 CC. |
|---|---|---|---|---|
| | | By gas analysis | From iron | |
| | | *cc.* | *cc.* | *mgm.* |
| 17–10–22 | 1 | 13.2 | 14.0 | 34.2 |
| 12– 9–22 | 5 | 13.0 | 13.6 | 34.1 |
| 19– 9–22 | 5 | 11.2 | 11.8 | 29.7 |
| 13–10 22 | 6 | 12.4 | 12.2 | 30.5 |
| 3–10–22 | 7 | 10.1 | 10.4 | 26.1 |

TABLE 4

*Seasonal changes in the gases and in the sugar of the blood*

| | | 1922 | | | | | | 1923 |
|---|---|---|---|---|---|---|---|---|
| | | Jan.-Feb. | Mar.-April | May-June | July-Aug. | Sept.-Oct. | Nov.-Dec. | March |
| Volume per cent: | | | | | | | | |
| Oxygen capacity | Mean | 13.7 | 10.6 | 9.5 | 9.9 | 11.8 | 12.2 | 15.3 |
| | St. dev. | 2.5 | 2.0 | 2.5 | 1.3 | 1.4 | 0.6 | 2.7 |
| Oxygen contents | Mean | 11.0 | 5.5 | 1.8 | 3.5 | 4.3 | 5.7 | 10.9 |
| | St. dev. | 1.5 | 1.4 | 0.2 | 1.6 | 1.2 | 2.7 | 1.0 |
| Carbon dioxide capacity | Mean | 49.9 | 67.8 | 53.2 | 48.3 | 47.3 | 45.9 | 54.3 |
| | St. dev. | 5.9 | 5.3 | 12.3 | 3.7 | 7.1 | 3.6 | 7.6 |
| Carbon dioxide contents | Mean | 38.6 | 60.6 | 48.6 | 44.4 | 40.7 | 42.6 | 49.4 |
| | St. dev. | 5.5 | 4.9 | 12.6 | 5.5 | 3.8 | 2.4 | 16.1 |
| Sugar in mgm. per 100 cc. of blood | Mean | 97.0 | 95.2 | 92.8 | 83.0 | 65.7 | 29.6 | 58.0 |
| | St. dev. | 27.7 | 23.6 | 21.5 | 26.4 | 11.8 | 10.8 | 10.2 |

Fry (27) has shown that of a lizard to be 3 per cent and of the frog to be 4.6 per cent of the body weight. If the blood volume of the alligator is assumed to be of this order, we have at no time drawn more than 12 per cent of the total blood volume, and as two weeks were allowed for recovery, it is felt that hemorrhage of itself was not a disturbing factor.

To check the accuracy of the method of gas analysis, the iron of the blood was estimated by the method of Brown (13). The oxygen capacity of the blood was then calculated from the iron and compared with that obtained by gas analysis. These figures are shown in table 3, and a satisfactory agreement is apparent.

*4. Seasonal changes of blood gases.* The mean values for the gases of the entire series, grouped in bi-monthly periods, are shown in table 4. A survey of the figures for oxygen capacity arranged in bi-monthly periods (table 4), shows that the oxygen capacity reaches a maximum in the early spring, and is lowest during the late spring and summer months. The value found in March of the second year is higher than that of the preceding summer, and is slightly higher (by about 5 volumes per cent) than that of the corresponding period of the preceding year. This increase is very probably due to the stimulation of the formation of hemoglobin due to repeated hemorrhage to which the animals had been subjected, and which is known to occur in birds and mammals (28).

The oxygen contents for the period of January and February have a value of 11.0 volumes per cent; in March and April of the same year of 5.5 per cent; and for May and June of 1.8 per cent, which is the lowest value observed. Thereafter there is a gradual increase until the value of 5.7 volumes per cent is attained in the November and December period. In the following spring the values are again high giving an average of 10.9 per cent for the series.

The oxygen unsaturation of the blood varies from 20 per cent during the January and February period through 58 per cent for March and April, to 81 per cent in May and June. From this it progressively decreased to 46 per cent for November and December. Determinations were not obtained during January and February of the second year but those made early in March gave a value of 29 per cent.

Any interpretation of these results must take into account the fact that the animals were kept at the approximate temperature of 26.5°C. No observations of the blood gases of the alligator in its natural habitat have been made but the differences we find during the year are such as we might expect to find if the animals were subjected to seasonal changes. In cold weather, the metabolism and consequent use of oxygen are at a low level and the oxygen unsaturation of venous blood would be slight. In summer months when metabolism is more rapid, we would expect the oxygen unsaturation also to be correspondingly greater, since the oxygen is consumed to a greater extent.

We must interpret these results as due to a periodicity acquired by the animal in its former habitat, or perhaps to some natural, internal rhythm of the animal, but certainly not to the immediate environment in which the animals lived during the period of observation.

The blood of different animals when exposed to carbon dioxide at a tension of 40 mm. of mercury shows a varying capacity for fixing this gas. The carbon dioxide capacity is distinctly higher in the early spring than during the remainder of the year. This is illustrated in table 4, from which it appears that the carbon dioxide of the March and April period is well above that of the other periods. The fact that the volume for the second March is lower than the corresponding earlier period is possibly due to a partial loss of the periodicity resulting from the prolonged period in which the animals were subjected to constant temperature conditions.

The carbon dioxide contents show a similar behavior. In the March and April period, carbon dioxide content is greater than in the winter or summer months. The lowest period seems to be in the fall and winter. In the second spring the value is higher than during the preceding winter and summer, but is not as high during the first spring. Animals 4,6 and 7 gave high values while the other three animals were low. The fact that some of the animals do not show an increase at this time of the year, while some do, is possibly due to a gradual loss of the periodicity due to constant conditions.

*5. Gaseous nitrogen of the blood.* The gaseous nitrogen of the blood was found to be higher than in any animal so far reported. In human blood, the average value for the nitrogen is 1.36 volumes per cent (29). In the horse, Regnard and Schlosing (30) found the nitrogen to be 2.00 volumes per cent. On ten determinations the mean value for the alligator was 0.033 with a standard deviation of 0.0055, on 1 cc. of blood, giving a value of 3.3 volumes per cent.

*6. Chlorides.* The chloride content of the blood of the alligator was found to be 525 mgm. per 100 cc. of blood. The value for dog's blood is about 550 to 570 mgm. per 100 cc.; for ox blood about 450 mgm., and for human blood 650 mgm. per 100 cc. The urine appeared to be wholly free from chlorides, not a trace being found at any time.

*7. Relative efficiencies of erythrocytes of alligator and man.* It was of interest to compare the relative efficiency of the erythrocytes of the alligator and man as oxygen carriers. This was done by comparing the actual size of the red corpuscles of the alligator with those of man, and at the same time, comparing each with respect to oxygen carrying power. From this it may be possible to form some idea of the physiological efficiencies of the cells of the two types of animal.

The volume of each erythrocyte may be calculated by dividing the corpuscular volume by the number of red cells. Thus in man, the corpuscular volume may be taken as 40 per cent (31) and the number of red cells per cubic millimeter as 5,500,000 (31). The volume occupied by one corpuscle would be 0.4 cu.mm. divided by 5,500,000 or 7.2 $\times$

$10^{-8}$ (32). In the alligator a wide range in the number of red cells was found, but the average number per cu.mm. is 850,000, and the average corpuscular volume per cent is 14.5. The volume of the individual corpuscle, therefore, is 0.145 cu.mm. divided by 850,000 or $16 \times 10^{-8}$. The alligator corpuscles therefore compare in volume with the human corpuscles as $\frac{16 \times 10^{-8}}{7.2 \times 10^{-8}}$ or as 2.2: 1.

The oxygen capacity of human blood is about 19 cc. per 100 cc. of blood. The factor for converting oxygen capacity into hemoglobin is 0.746. The hemoglobin content of human blood is 14 to 15 grams per 100 grams of blood. The oxygen capacity of alligator blood may be taken as 12 cc. per 100, and the hemoglobin content would then be about 9 grams per 100 grams of blood. If a proportional amount of hemoglobin were carried by the two kinds of erythrocytes, the corpuscular volume would also be proportional. That is 9: 14.5 as X: 40. The corpuscular volume of the alligator under these circumstances would be about 25 volumes per cent. The corpuscular volume was found, however, to be 14.5 per cent and thus 25/14.5 represents the ratio between the hemoglobin carrying powers of the two kinds of corpuscles. The alligator corpuscles then either contain relatively more hemoglobin than human corpuscles, or in some way the hemoglobin is more efficient. From this we may conclude that one alligator corpuscle is equal in volume to 2.2 human corpuscles and in its efficiency as an oxygen carrier is 1.7 times that of the human corpuscle. So it would take about four human erythrocytes to equal one alligator corpuscle as an oxygen carrier.

*8. Comparison of the gases of arterial and venous blood.* In order to compare the gaseous content of arterial and venous blood, samples were drawn successively in the manner described above, with the minimum possible time interval between samples, since it was obviously impractical to draw the two samples simultaneously. This was done, perhaps unfortunately, at the season of the year when the animals showed the maximum variation between individuals. However the results are interesting and are included as the first observations of the kind upon the alligator.

The detailed results are shown in table 5. The carbon dioxide contents, given by alligator 4, which alone gave the average value for this season as shown in table 4, for venous blood was 38.4 volumes per cent, and 32.0 volumes per cent for arterial blood, a difference of 6.4. Similarly, the oxygen content of venous blood was 11 volumes per cent and for arterial blood 15.6 volumes per cent, and the difference was 4.6.

Similar data are not available for many species though Buckmaster and Gardner (33) publish some figures for the cat, and Hastings (34),

among others, for the dog, while there are, of course, many observations upon man (35). For purposes of comparison some of these data are included in table 6. It must be borne in mind that values of this kind are never absolute but must vary with the nature of the metabolism of the several individuals at the time that the samples were taken.

Peters, Barr and Rule (36) quote respiratory quotients in human blood, of 1.62 to 0.34, and Harrop (37) reports values of 1.81 to 0.62.

*9. Carbon dioxide absorption curve.* Oxalated blood was exposed to atmospheres containing known percentages of carbon dioxide and air. Samples were removed and analyzed after equilibrating the blood with

TABLE 5

*Arterial and venous blood of the alligator*

| NUMBER OF ANIMAL | DATE | VENOUS | | ARTERIAL | | DIFFERENCE | | R.Q. |
|---|---|---|---|---|---|---|---|---|
| | | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2/O_2$ |
| 3 | 2-16-23 | 33.0 | 10.5 | 25.8 | 13.7 | 7.2 | 3.2 | 2.2 |
| 4 | 2-16-23 | 38.4 | 11.0 | 32.0 | 15.6 | 6.4 | 4.6 | 1.4 |
| 6 | 2-13-23 | 66.5 | 10.0 | 59.1 | 14.6 | 6.5 | 4.6 | 1.43 |
| 7 | 2-15-23 | 48.3 | 4.1 | 41.1 | 10.1 | 7.2 | 6.0 | 1.2 |
| Mean | | | | | | 6.8 | 4.6 | 1.47 |

TABLE 6

*Gases in volumes per cent in arterial and venous blood of several animals*

| | VENOUS | | ARTERIAL | | DIFFERENCE | | R.Q. |
|---|---|---|---|---|---|---|---|
| | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2/O_2$ |
| Human | 48.0 | 12.0 | 40.0 | 20.0 | 8.0 | 8.0 | 1.00 |
| Dog | 40.5 | 14.3 | 38.3 | 19.3 | 2.2 | 6.0 | 0.37 |
| Cat (etherized) | 40.8 | 9.9 | 25.07 | 13.6 | 15.77 | 3.7 | 4.2 |
| Alligator 4 | 38.4 | 11.0 | 32.0 | 15.6 | 6.4 | 4.6 | 1.4 |

the gaseous mixtures. The results are plotted in figure 2, by placing tensions (calculated from percentages) of carbon dioxide in millimeters of mercury, on the abscissae and volumes per cent of carbon dioxide absorbed by the blood, on the ordinates. Determinations of this kind were made on the blood of each of three animals. The three curves fall at different heights as would be expected from the differences in alkali reserve already noted. They are, however, approximately parallel and the middle curve does not depart greatly from that for human blood published by Christiansen, Douglas and Haldane (38), which is drawn from the mean of many observations and which is represented by plus marks (+) in the figure. From this one might conclude that the mechanism of carriage of carbon dioxide in the blood of the alligator is essentially the same as that of human and mammalian blood, in general.

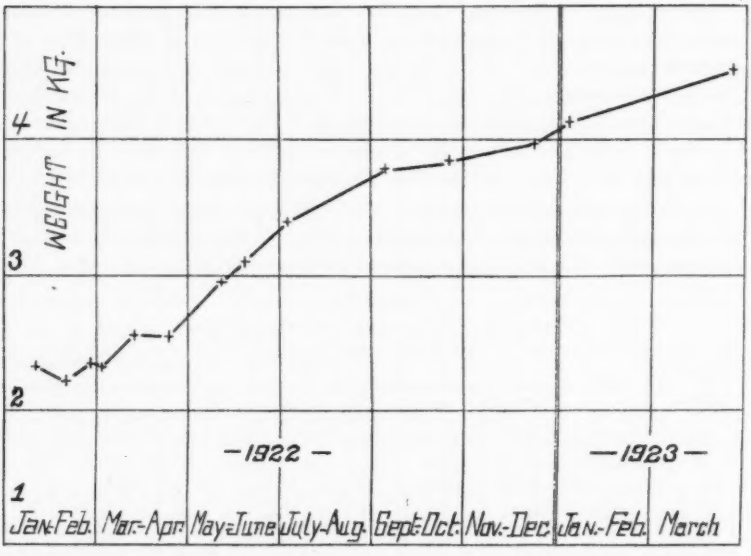



Fig. 1. Weight curve for alligator 1. The curves for the other individuals were essentially parallel to this. Only in two cases did the curves of the different individuals cross.

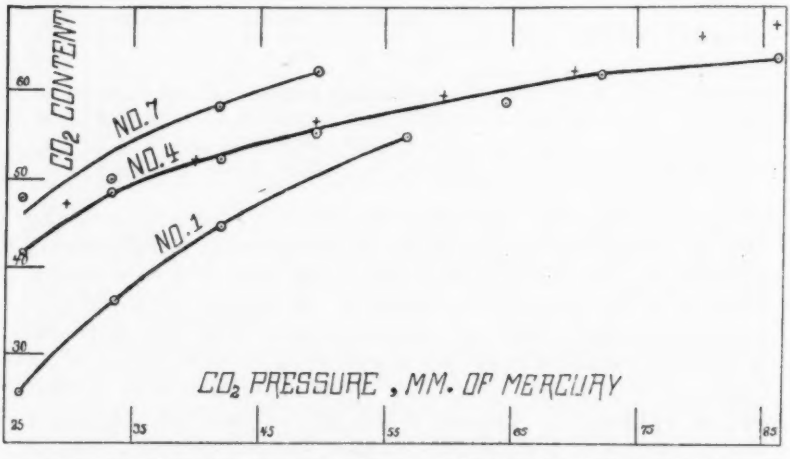



Fig. 2. Carbon dioxide dissociation curve for the blood of three alligators (circles) compared with that for human blood (crosses).

It might be further noted that the variations in alkali reserve among the individuals at this season are no greater than those found by Hastings (34) for different dogs.

*10. Seasonal changes in the concentration of sugar in the blood.* The concentration of the sugar in the blood of these animals was determined throughout the year. The mean values arranged in bi-monthly periods are shown in table 4. Animal 3, though subjected to the same general conditions, was used very little in the routine work of the study and served in the nature of a control on the disturbances due to the experimental procedure. The blood sugar in this animal was 97 mgm. per 100 cc. of blood in the January–February period, 92 mgm. in the July–August period, and 59 mgm. in the November–December period. In March of the second year the value was 92 mgm., about the same level as at the beginning of the experiment. The reaction of this animal was of the same nature as that of the other animals, but more marked, as will be seen upon comparison of the figures of this animal with those given in table 4.

A high sugar content is shown by all of the animals during the late winter and early spring. During the late spring a distinct drop in the sugar is noted, which continues during the summer and autumn, the lowest values being obtained for the November and December period. At this time, the mean for all the animals was only 37.5 mgm. per 100 cc. of blood.

Since the earlier determinations gave an average of about the same magnitude as is common in the mammals, i.e., 97 mgm., it is of interest to compare the low value with the "critical value" of 44 mgm. described by Banting, Best, Collip, Macleod and Noble (39). It should be noted that during this "low" period the alligators appeared in every way normal and so far as one could determine by appearance, were in the same physiological conditions as when the blood sugar was at its highest. The work reported by Riddle and Honeywell (40) for the pigeons indicates that the blood sugar of at least some forms can be reduced to a very low level indeed (25 to 30 mgm.) without evoking the disturbances which have been described for those of the mammals which have been studied. Alligator 1, on November 21, 1922, showed no sugar in its blood, though at the same time it appeared otherwise to be in normal condition, and on March 7, 1923, yielded a value of 113 mgm. This reminds one of the behavior of the dogfish where the sugar was at times low or absent. (See Scott (41) and Macleod (42).)

The annual cycle in the above discussed data is of much interest from the standpoint of seasonal changes in physiological phenomena, but any interpretation can only be conjectural at the present time.

The work of Riddle and Honeywell (43) shows a periodicity in the accumulation of sugar in the blood of the pigeon accompanying the periodicity of ovulation. The period of ovulation in the alligator occurs only in June, and only animals of mature age (five to ten years) carry out this function (44). All of our animals showed this seasonal rhythm, even the smallest which was hardly two years old, so in this case the periodicity in the blood sugar is evidently not related to ovulation.

The periodicity may be related to the storage of glycogen shown to occur during autumn and winter in the marmot by Claude Bernard (45) and in the frog by Moseik (46), since we found the sugar to be low at the season of the year that the glycogen in these animals is high.

In plants an accumulation of reserve substances occurs during the fall and winter, and a mobilization of these in a diffusible form occurs in the late winter and early spring (47), and in this respect may resemble a poikilothermic animal that passes through a winter period of lowered metabolism.

In all cases where the behavior of these alligators is related to hibernation, it is necessary to remember that they must have carried their periodicity over from their former habitat which was northern Florida, and hence our late winter period would correspond to their early spring.

In addition to any such conjectural considerations it is of course necessary to add the possible effects of the temperature changes in concentration of the blood sugar which may be presumed to be the results of such more immediate physiological equilibration.

*11. Discussion of seasonal changes.* There has grown up a very considerable literature on seasonal variations of physiological functions in various animals. In this literature it is developed that there are very few if any organs or physiological systems that are not affected by this factor in one or another of the animal groups. This is not the time for an extensive reivew of the literature though a brief review of some of the papers on variations in the respiratory system, the metabolism of the carbohydrates and the persistence of the seasonal variations after the animals have been removed from their natural and variable environment to the more or less perfectly controlled conditions of the laboratory may not be out of place. From the foregoing discussion it will be remembered that there was a periodicity in the concentration of the gases and the sugar of the blood of the alligator, which must be related to previous effect of changes in the seasons of the year, and not to the immediate environment of the animal.

The respiratory exchange in the lungs of the frog under laboratory conditions was found by Athanasiu (59) to varying according to the season of the year. He found that the respiratory quotient was greater in the winter months than in the summer.

During hibernation of the woodchuck, Rasmussen (48) found a gradual increase in the amount of carbon dioxide in venous blood from about 70 volumes per cent to as high as 100 volumes per cent. His results supported those of Dubois (49). When the animal wakes from its winter sleep the carbon dioxide falls very rapidly to its lower level.

In the frog there is a seasonal change in the storage of glycogen in the liver and other organs of the body. Mozeik (46) has shown that in summer there is scarcely a trace of glycogen in the liver, but in the late autumn the liver is extremely rich in glycogen. This observation was confirmed by Athanasiu (50). The period of high glycogen content in the frog corresponds to the period of low sugar content of the blood in the alligator.

TABLE 7

*Nitrogen distribution in the urine*

| NUMBER OF ANIMAL | DATE | SP. GR. | MGM. PER 100 CC. | | | | PER CENT TOTAL N | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | N | $NH_3$ | Urea-N | Uric acid-N | $NH_3$ | Urea | Uric acid |
| 2 | 7–27 | 1.005 | 219 | 171 | 7.0 | 39.0 | 79.5 | 3.3 | 17.0 |
| 2 | 7–26 | 1.0062 | 249 | 198 | 3.3 | 28.0 | 80.0 | 1.3 | 11.0 |
| 1 | 8–4 | 1.0063 | 225 | 148 | 13.0 | 24.2 | 66.0 | 5.8 | 10.8 |
| 5 | 8–7 | 1.0048 | 268 | 208 | 16.0 | 18.4 | 77.0 | 6.0 | 7.0 |
| After sterile catheterization | | | | | | | | | |
| 1 | 8–14 | 1.0049 | 208 | 168 | 0 | 41.3 | 81.0 | 0 | 19.8 |
| 1 | 11–8 | | 207 | 140 | 20.5 | | 67.0 | 10.0 | |
| 1 | 11–9 | 1.0053 | 202 | 154 | 35.0 | | 77.0 | 17.0 | |

Claude Bernard (45) showed that in hibernating animals there is an increase in glycogen up to and including the period of winter sleep. The glycogen rapidly disappears when the animal awakes.

Physiological processes may be modified by a diurnal or annual cycle independently of the experimental conditions.

*12. Distribution of the non-protein nitrogen in the blood and urine.* The urine was collected by direct catheterization of the cloaca. The evidence that the urine so obtained was not contaminated by the feces and was in fact pure urine consists of the fact that it was always found to be a clear, limpid fluid, and that the specific gravity was found to be as consistent as would be expected. See table 7.

Analysis of the nitrogenous constituents of the urine always showed a high percentage of ammonia, in all cases more than 50 per cent of the total nitrogen appeared in this form, and occasionally more than 85 per cent.

Urea was usually present, in amounts varying up to 17 per cent of the total nitrogen, but was occasionally absent. Extra precautions for sterile catheterization tended to increase the proportion appearing in this form, but not in significant amounts.

Seven to 20 per cent of the total nitrogen of the urine appears as uric acid. The low percentage of uric acid is of interest in view of the common statement that the nitrogen metabolism of the reptiles is similar to that of the birds.

Wollaston in 1810 (51) showed that uric acid occurs in large amounts in the excrement of birds. Since this time, it has been shown by the investigation of a large number of workers that uric acid in birds arises from *a*, lactic acid and ammonia; *b*, from amino acids; *c*, from the purine bases, and *d*, from urea. Hoppe-Seyler showed that the excrement of certain lizards may consist almost wholly of uric acid (52), and Salkowski (53) mentioned the excrement of most snakes as a convenient source of

TABLE 8

*Uric acid in blood and urine*

| NUMBER OF ANIMAL | BLOOD | | URINE | | |
|---|---|---|---|---|---|
| | Date | Mgm. uric acid per 100 cc. | Number of animal | Date | Mgm. uric acid per 100 cc. |
| 1 | 4–20 | 1.67 | 1 | 7–25 | 36.8 |
| 1 | 5–11 | 2.25 | 1 | 7–26 | 37.6 |
| 4 | 5–4 | 2.56 | 1 | 8–2 | 27.8 |
| 5 | 4–27 | 1.83 | 1 | 8–3 | 23.6 |
| 5 | 5–18 | 2.60 | 1 | 8–4 | 24.2 |
| 6 | 5–9 | 1.79 | 1 | 8–14 | 41.3 |
| 7 | 5–16 | 2.24 | 1 | 10–24 | 22.8 |

obtaining uric acid in the pure form. The excrement of certain Pythons was shown to consist of ammonium urate (54). Snakes and lizards appear, therefore, to be similar to the birds in regard to the main end product of their protein metabolism. That this is not true of all forms of reptiles is shown, however, by the work of Karr and Lewis (55), who found large quantities of urea in the blood of the turtle. It is not true of the lower vertebrates, for nitrogen is largely eliminated by frogs (56) and fish (57) as urea. The facts just cited have given rise to the generalization that animals living in dry habitats, birds, snakes, lizards, excrete nitrogen as uric acid, while those living in water, fish, frogs and turtles, excrete it as urea instead. To this latter list we had hoped to add the alligator, but all we can say at the present time is, that it does not excrete large amounts of uric acid, and may possibly take an intermediate position between the lizards and the turtles.

The urine was neutral or faintly alkaline on drawing, but rapidly became strongly alkaline on standing, and a crystalline deposit of calcium carbonate and triple phosphates was thrown down. When collected under oil the urine showed a very high content of carbon dioxide, as much as 250 cc. per 100 cc. of urine. The change of reaction on standing is probably due in part to loss of $CO_2$.

We have found nothing in the literature on the nitrogen of the blood of the alligator, although Krukenberg (58) found creatine and hypoxanthin in the muscle extract of the alligator. We had hoped that analysis of the nitrogen constituents of the blood would throw light on the origin of the large quantities of ammonia in the urine. Contrary to our expectation, no urea was found in the blood. That this was not due to faulty technic is indicated by the fact that known amounts of urea added to the blood were recovered, and that the accepted values were found for beef blood. Less than 15 per cent of the non-protein nitrogen of the blood is in the form of ammonia. In other cases, where uric acid

TABLE 9

*Nitrogen distribution in the blood*

| NUMBER OF ANIMAL | DATE | MGM. PER 100 CC. | | | PER CENT TOTAL N |
|---|---|---|---|---|---|
| | | Non-protein N | $NH_3$ | Urea | $NH_3$ |
| 2 | 1–4 | 15.0 | 2.1 | 0.7 | 14.0 |
| 1 | 1–5 | 15.0 | 2.1 | 0 | 14.0 |
| 7 | 1–9 | 19.6 | 2.1 | 0 | 10.7 |
| 4 | 1–10 | 18.0 | 2.1 | 0 | 11.7 |
| 6 | 1–11 | 16.0 | 2.1 | 0 | 13.0 |

alone was determined, the quantity of uric acid was found to average 2.13 mgm. per 100 cc. of blood, which represents only about 4 per cent of the non-protein nitrogen.

Neither creatine nor creatinine were found in either blood or urine.

The total nitrogen of the blood was found to be about 2000 mgm. per 100 cc. of blood.

The apparent absence of urea and the relatively small quantity of ammonia in the blood may indicate that there is some unknown precursor of the urea and ammonia which is found in the urine. The small amount of uric acid present in the blood would indicate that this precursor is probably not uric acid.

I wish to express my great appreciation of the continual assistance given me by Professor E. L. Scott of this Department, in carrying out this investigation.

SUMMARY

1. The erythrocytes of the alligator average 850,000 per cu.mm. of blood. They are about 14 $\mu$ in width and about 25 $\mu$ long, and are oval, nucleated cells.

2. The average corpuscular volume is about 14 to 15 per cent.

3. The oxygen capacity of the blood varies according to the season of the year between the values 9.5 and 13.7 volumes per cent. The oxygen content varies from 1.8 to 11 volumes per cent. The oxygen unsaturation of the venous blood is slight in late winter (20 per cent) and is very great in the summer (80 per cent).

4. The iron content of the blood is about 30 mgm. per 100 cc.

5. The carbon dioxide capacity and content of blood also show a seasonal variation, being about 20 volumes per cent higher in early spring than during the summer and winter.

6. The carbon dioxide absorption curve of alligator blood is essentially the same as that of human blood.

7. The sugar content of the blood shows a seasonal variation, being high in the spring and very low during late fall and winter.

8. Uric acid is present in only small quantities in the blood and urine of the alligator.

9. The main nitrogen constituent of the urine is ammonia. The precursor in the blood of the ammonia in the urine is not known.

BIBLIOGRAPHY

(1) VERNON: Journ. Physiol., 1897, xxi, 443.
(2) FLINN AND SCOTT: This Journal, 1923, lxvi.
(3) MINKOWSKI: Arch. f. exper. Path. u. Phar., 1886, xxi, 54.
(4) KNIERIEM: Zeitschr. f. Biol., 1877, xiii, 36.
(5) MEYER UND JAFFE: Ber. d. deutsch. Chem. Gesell., 1877, x, 1930.
(6) SCHRÖDER: Zeitschr. f. Physiol. Chem., 1878, ii, 228.
(7) MACH: Arch. f. exper. Path. u. Phar., 1888, xxiv, 399.
(8) SALKOWSKI UND LEUBE: Die Lehre von Harn. 1882, 87.
(9) VAN SLYKE AND CULLEN: Journ. Biol. Chem., 1917, xxx, 289.
(10) TIEGEL: Arch. f. Physiol. von Pflüger, 1880, xxiii, 278.
(11) VAN SLYKE AND STADIE: Journ. Biol. Chem., 1921, xlix, 46.
(12) VAN SLYKE: Journ. Biol. Chem., 1918, xxxiii, 127.
(13) BROWN: Journ. Amer. Chem. Soc., 1922, xliv, 423.
(14) MACLEAN: Biochem. Journ., 1919, xiii, 135.
(15) HASTINGS AND HOPPING: Proc. Soc. Exper. Biol. and Med., 1923, xx, 254.
(16) Organic Analysis. Official Methods. Baltimore, 1916, 5.
(17) MYERS: Practical chemical analysis of blood, 1921, 34.
(18) FOLIN: Journ. Biol. Chem., 1919, xxxviii, 81.
(19) BENEDICT: Journ. Biol. Chem., 1922, li, 187.
(20) VAN SLYKE AND CULLEN: Journ. Biol. Chem., 1914, xix, 211.
(21) STEELE: Journ. Biol. Chem., 1910, viii, 365.
(22) BENEDICT: Journ. Biol. Chem., 1922, lii, 387.

(23) AUSTIN AND VAN SLYKE: Journ. Biol. Chem., 1920, xli, 345.
(24) HARVEY: Arch. Int. Med., 1910, vi, 12.
(25) REESE: American alligator. New York, 1915.
(26) WHIPPLE: Harvey Lecture, 1922.
(27) FRY: Quart Journ. Exper. Physiol., 1913–14, vii, 185.
(28) DOUGLAS: Journ. Physiol., 1906, xxxiv, 210.
(29) VAN SLYKE AND STADIE: Journ. Biol. Chem., 1921, xlix, 1.
(30) REGNARD AND SCHLOESING: Compt. rend. Acad., 1897, cxxiv, 302.
(31) MATTHEWS: Physiological chemistry. New York, 1920, 475, 990.
(32) STARLING: Human physiology, 1921, 3rd ed., 861.
(33) BUCKMASTER AND GARDNER: Journ. Physiol., 1910, xli, 60.
(34) HASTINGS: Pub. Health Bull., 1921, no. 117.
(35) MACLEOD: Physiology and biochemistry, 1922.
(36) PETERS, BARR AND RULE: Journ. Biol. Chem., 1921, xiv, 489.
(37) HARROP: Journ. Exper. Med., 1919, xxx, 241.
(38) STARLING: Human physiology, 3rd ed., 1116.
(39) BANTING, BEST, COLLIP, MACLEOD AND NOBLE: Trans. Roy. Soc. Can., 1922, xvi, Sect. 1–16.
(40) HONEYWELL AND RIDDLE: Proc. Soc. Exper. Biol. and Med., 1923, xx, 248.
(41) SCOTT: This Journal, 1921, liv, 262.
(42) LANG AND MACLEOD: Quart. Journ. Exper. Physiol., 1920, xii, 331.
(43) HONEYWELL AND RIDDLE: Proc. Soc. Exper. Physiol. and Med., 1922, xix, 377.
(44) REESE: American alligator, 1915.
(45) BERNARD: Compt. rend. Acad. Sci., xlviii, 673.
(46) MOSEIK: Arch f. Physiol. von Pflüger, 1888, xlii, 556.
(47) MICHEL: Dissert. Göttingen, 1909, 43.
(48) RASMUSSEN: This Journal, 1916, xxxix, 20; also xli, 162.
(49) DUBOIS: Physiologie Compareé de la Marmotte. Paris, 1896, 79.
(50) ATHANASIU: Arch. f. Physiol. von Pflüger, 1911–12, lxxiv, 561.
(51) WOLLASTON: Annal. de Chem., 1810, lxxvi, 31.
(52) HOPPE-SEYLER: Med. Chem. Untersuch., 1871, iv, 584.
(53) SALKOWSKI UND LEUBE: Die Lehre von Harn, 1882, 87.
(54) BACON: Phill. Journ. Sci., 1909, iv.
(55) KARR AND LEWIS: Journ. Amer. Chem. Soc., 1916, xxxviii, 1615.
(56) NEBELTHAU: Zeitschr. f. Biol., N.F. 1889, vii, 129.
(57) DENIS: Journ. Biol. Chem., 1922, liv, 693.
(58) KRUKENBERG: Vergleichen-Physiologische Studien, 1882. II Reihe. II Abt.
(59) ATHANASIU: Arch. f. Physiol. von Pflüger, 1900, lxxix, 400.

# STUDIES IN THE PHYSIOLOGY OF VITAMINS

## II. Parenteral Administration of Vitamin-B—Mammalian Experiments

GEORGE R. COWGILL

*From the Sheffield Laboratory of Physiological Chemistry in Yale University, New Haven, Conn.*

Received for publication June 16, 1923

Investigations into the physiology of vitamin-B, using mammals as experimental animals, have been fairly extensive. The rat, mouse, guinea pig, pig, goat, monkey and the dog have been utilized for such studies. Such use of these mammals has involved either *a*, an attempt, by feeding a one-sided diet consisting in most cases of milled rice, to produce in the experimental animals symptoms resembling those characteristic of human beriberi; or *b*, carefully controlled studies in nutrition where diets adequate as far as possible except with respect to a single factor, namely, vitamin-B, were fed.

It has long been known that the paralytic symptoms characteristic of vitamin-B deficiency in the pigeon may be relieved in a most remarkable manner when a sufficient amount of some source of the vitamin is administered parenterally. Recoveries in birds have been reported as occurring in as short a time as from one to four hours (21). Reports have also been made by many investigators working with fowls as experimental animals (19), (10) and confirmed by the writer's experience, that the administration of vitamin-B by way of the crop to avitaminotic pigeons results either in a slow recovery, many hours elapsing before improvement is noticed, or in a complete failure, the bird being unable to swallow the material given due to a paralysis of this organ.

Such results indicate that a marked difference in the curative power of a vitamin-containing preparation exists when administration *per os* is compared with treatment by parenteral paths. It becomes of interest, therefore, to inquire whether this is true of such an animal as the dog in which characteristic symptoms due to lack of vitamin-B may be induced by feeding the animal on an artificial mixture of isolated food substances adequate except with respect to this antineuritic vitamin.

A review of the literature does not indicate that parenteral administrations of vitamin-B to *mammals* have ever been made.

De Lacerta (3) made pathological studies of a disease prevalent among horses and hogs in Brazil and pointed out its similarity in many respects to beriberi. This author's paper reports the findings obtained from examination of the tissues of animals which had died from this disease; no experiments with the view to curing such cases were made.

According to Schaumann (16), Grijns (5), (6) fed dogs with autoclaved lean meat and the animals manifested beriberi symptoms. This same investigator (7) showed that unpolished rice may lose its protective power against such symptoms when heated to 130°C. (cited by Gibson and Concepcion (4)). Parenteral administrations of the curative agents do not appear to have been made in these experiments.

At a symposium on *Beriberi*, Hose (8) mentioned experiments which he had performed on three monkeys (*Macacus nemestrinus*). The animals were fed on bazaar rice, two of them developing some of the characteristic symptoms of beriberi.

In his book on *The Cause and Prevention of Beriberi*, Braddon (1907) describes a disease prevalent among the horses of India which are fed a dietary consisting for the most part of rice paddy (cited by Voegtlin and Lake (20)). Experimental studies of this condition are lacking however.

The original papers of Shiga and Kusama (17) who worked with monkeys, and of Schaumann (16) who used rats, guinea pigs, a dog, a goat and a monkey for his studies, are not available to the writer and therefore have not been consulted for details regarding the experiments. However, in view of the accounts of these experiments given by Vedder (19), it does not appear that parenteral administrations of sources of vitamin-B were made. These investigators seem to have been interested primarily in bringing about by dietary means the production in their animals of symptoms resembling human beriberi, and in a study of the pathological changes induced in the tissues of such animals.

McCarrison (11) has experimented with monkeys and pigeons and studied the tissue changes brought about in such animals by feeding deficient diets, but has not devoted any attention to the methods by which curative agents may be administered.

Beriberi-like symptoms have been observed in pigs fed deficient diets (12), (15), but parenteral administrations of the missing dietary essential to such animals have not been reported.

Suzuki, Shimamura and Odake (18) experimented with their base *Oryzanin* prepared from rice polishings using both fowls and mammal as test animals. Their protocols indicate quite clearly that the mammals so used were given the curative base by mouth.

Gibson and Concepcion (4), Voegtlin and Lake (20) and Karr (9) experimented with dogs and likewise administered the vitamin-containing products by way of the alimentary tract.

The vitamin-B literature concerning the smaller mammals such as the mouse and rat is very extensive; if any accounts of parenteral administration of products containing vitamin-B to such animals have been published, they have escaped the attention of the writer.

The behavior of dogs which are fed various synthetic diets adequate except with respect to vitamin-B has already been described (9), (1).

That the peculiar complex of symptoms exhibited in such animals is truly a result of vitamin-B deficiency appears to be quite evident upon consideration first, of the fact that it does not develop when the animals are given a daily supplement to the diet of a sufficient amount of some source of the vitamin; and second, that it can be relieved when liberal quantities of a source of vitamin-B are given to the animal by means of a stomach tube. Administration of the missing dietary essential by this method is necessary in most cases because the animals have lost all desire to eat and because most of the available sources of vitamin-B are mixtures of many substances including protein, and therefore do not lend themselves for use in administration by parenteral channels. Furthermore, dogs suffering from vitamin-B deficiency usually show vomiting as one feature of the symptom complex and generally require several treatments with vitamin-containing material by stomach before relief can be brought about. In all recoveries of this type, occurring in the writer's experience, at least fifteen to eighteen hours have elapsed before relief was quite apparent, and in many cases of a more advanced type where severe convulsions and clonic spasms had appeared, administrations of liberal amounts of the vitamin-containing material by stomach were without effect, such cases terminating fatally.

If parenteral administration of a suitable source of vitamin-B to such dogs were made and rapid recoveries obtained similar to those occurring in polyneuritic pigeons thus treated, it would be additional proof that this pathological condition in the dog is the result of a vitamin-B deficiency. *Such experiments have now been performed:* they afford striking confirmation of the contention that the pathological condition of the animals which were described in a previous paper (1) and which occurred when the dogs were fed on artificial mixtures of isolated food substances adequate except with respect to vitamin-B, is caused by the absence of this accessory from the diet. Furthermore, this condition is an uncomplicated one occurring in so large an animal as the dog and, therefore, may be made the subject of many types of study having as their object the elucidation of the physiological rôle played by the antineuritic vitamin.

EXPERIMENTAL. *Vitamin-B preparation used. Yeast vitamine powder (Harris)*[1] was the source of vitamin-B used for these experiments. This material, prepared by Dr. I. F. Harris and kindly donated by him for use in these experiments, is made from yeast following the general directions of Osborne and Wakeman (14) together with a few modifications in procedure introduced by Doctor Harris. It is protein-free and, so far as tests upon young rats indicate, represents

[1] From the Harris Laboratories, Tuckahoe, New York.

a concentration of from probably seven to ten times the vitamin-B contained in the brewery yeast from which it is made. The fact that this product is protein-free and available in large quantity suggested the line of experimentation reported in this paper.

All of the experimental animals were fed on diet III (table 1) until pathological symptoms appeared. The Yeast Vitamine Powder (Harris) was then administered by intravenous, intraperitoneal or subcutaneous injection and the effect of such injection noted.

The Harris vitamine proved to be soluble in water, such solution giving an acid reaction. In order to preserve to a high degree the potency of the original preparation it was deemed advisable to make the solution of the powder as shortly as possible before it was needed

TABLE 1

*Diet III*

| | GRAMS | CALORIES | PER CENT | |
|---|---|---|---|---|
| Casein comm'l (12.7% N) | 6.3 | 20.6* | 41.2 | |
| Sucrose | 4.5 | 18.0 | 29.4 | |
| Lard | 2.8 | 25.2 | 18.3 | |
| | | | | 25.5 |
| Butter | 1.1 | 9.0† | 7.2 | |
| Bone ash | 0.4 | | 2.6 | |
| Salt mixture‡ | 0.2 | | 1.3 | |
| Total | 15.3 | 72.8 | 100.0 | |

This kilo-unit contains 0.8 gram of N, and 73 calories, 46.5 per cent of which are furnished by fat.

* Calculated on a basis of 81.9 per cent protein.

† Figured as containing 90 per cent fat.

‡ Used by Karr (9).

for the experiment. Upon neutralizing this solution to litmus a precipitate appeared. After many experiments with filtering through paper and cotton, centrifugation was adopted as a standard procedure, largely because it made possible a rapid and sharp separation of solution from precipitate. From five to seven minutes' centrifugation at high speed proved to be sufficient for this purpose. The supernatant fluid thus obtained was decanted, sterilized by *boiling* gently in a covered beaker for five minutes, cooled and then injected into the animal. Using this general procedure material suitable for injection could be prepared in a short time—from one-half to one hour. This treatment undoubtedly diminished the potency of the original powder as an experiment to determine this clearly demonstrated. (See the description of experiments with dog 48 in this paper.)

*Intravenous injection.* Three dogs (nos. 41, 42 and 37) were treated by intravenous injection. Dog 41 received 10 grams of the Harris powder treated as previously described, by the jugular vein. At the time of the injection this animal had severe clonic spasms involving nearly all muscles, these spasms being particularly severe when the dog was handled; all of the limbs were in extension, and the head was raised approximating an opisthotonic position. A photograph of this animal was taken during such a spasm.

Fig. 1. Dog exhibiting nervous and muscular symptoms of vitamin-B deficiency. The animal is unable to stand and is seized with sever clonic spasms particularly when handled. The muscles are contracted resulting in extension of the limbs and in a raising of the head. After being photographed this animal received vitamin-B by intravenous injection.

Fig. 2. Same dog as in figure 1, photographed four hours and ten minutes after receiving vitamin-B treatment via the jugular vein. The muscles are relaxed and the animal no longer exhibits clonic spasms when handled; the dog is also able to walk, the only manifestation of vitamin-B deficiency now apparent being a moderate spasticity of the muscles of the hind limbs resulting in a spastic gait.

One hour after the injection the dog showed a few spasms of slight severity when handled, and a slight improvement in the control of its limbs A second photograph of this dog was taken four hours and ten minutes after the injection; this is reproduced in figure 2.

At this time the muscles were completely relaxed, the animal was able to walk easily, with but a slight spasticity of gait. While the second photograph was being made, the dog was supported as indicated in the picture in order to show more clearly the relaxed condition of the muscles, which was such a contrast to their appearance only four

hours before. This animal subsequently recovered its appetite for the same food upon which it had subsisted and was placed upon another experiment in which it received a daily supplement of a small amount of the Harris powder; under such conditions it thrived for one hundred and fifty-one days at which time the experiment was discontinued, and the animal taken off the artificial food mixture.

Dog 42 likewise exhibited clonic spasms and received 10 grams of the Harris vitamine by the jugular vein but died fifteen minutes after the injection had been finished. An autopsy made immediately gave no clue as to the cause of death. One finding of interest which merits further investigation proved to be the discovery of a large amount of bloody exudate in the peritoneal cavity. Since sudden death due apparently to heart failure is of occasional occurrence among dogs which exhibit nervous and muscular symptoms due to lack of vitamin-B, it is not unlikely that this animal died from a sudden cardiac breakdown.

Dog 37, which was a half-grown puppy, received an intrajugular injection of the Yeast Vitamine Powder (Harris) on the thirty-fourth day of feeding on the vitamin-free diet, when convulsions appeared. An extract from the protocol is given below.

*Extracts from protocol of dog 37.* January 27th—34th day. Dog vomited at about 9 a.m. at least 18 hours after having eaten food. Vomitus contained considerable mucus but no food particles. Animal had a prolonged vomiting spell. At about 5:15 p.m. the dog fell over with convulsions (clonic spasms involving all four limbs); when assisted to its feet, it swayed for a moment, attempted to walk, fell over on its side and exhibited another convulsion. At 6:40 p.m. dog received 9 grams of Harris powder, lot 965—neutralized and centrifuged—by jugular vein. Injection finished at 6:50 p.m. Dog was placed on its feet; it walked to the end of its cage and sat on its haunches. Dog was then placed in the middle of the room; it walked with a slightly spastic gait which became worse with each succeeding step, finally resulting in a convulsion which lasted about 5 seconds. Dog was placed in a box with burlap and the box placed near a warm radiator. At 7:15 p.m. the dog walked around the room with a scarcely perceptible spastic gait. At 7:25 dog appeared normal except for a slight spasticity of the hind limbs. Pulse 80, and respiration 40 per minute.

At 8:40 p.m., dog resting easy, pulse 120, respiration 40.

At 10:30 p.m., dog resting easy, pulse 84, respiration 36.

At 11:00 p.m., dog resting easy, pulse 100, respiration 36.

At 11:30 p.m., dog walked to cage (from radiator) and drank what appeared to be an excessive amount of water. The spastic gait was fairly marked. Dog was somewhat distressed, groaning with each respiratory movement.

January 28th—35th day. 8:30 a.m., dog is resting easy. Passed black feces during night; feces gave positive benzidene test for occult blood. 5:30 p.m., pulse is 132. Food left in cage all night.

January 29th—36th day. Dog ate 73 grams of food during the night. Passed black feces during the night. Is a much improved animal. Ate 32 grams of food during the forenoon. Has no bad breath.

January 30th—37th day. Dog is lively; eats all food offered in a few minutes. Has no bad breath.

January 31st—38th day. Same as 37th day.

As the above protocol indicates, the convulsions were relieved a half-hour after finishing the injection This recovery, therefore, was even more striking than that obtained with dog 41, but an exact comparison of these two cases is not possible because there is no way by which we can determine whether the conditions in these animals were of equal severity The spastic condition of the limb muscles of these dogs was not abolished so promptly; the spastic gait was quite apparent for af least six hours.

In the light of these experiments it appears that *the severe convulsions which occur in dogs suffering from vitamin-B deficiency may be relieved in a very striking fashion within the course of a few hours when a sufficient amount of the vitamin is injected intravenously.*

*Intraperitoneal injection.* Four dogs (nos. 34, 35, 36 and 43) were given the Harris vitamin preparation by the intraperitoneal route. Three of these animals were suffering from a lack of vitamin-B, whereas the fourth dog was in a healthy condition, it being given an injection in order to determine the effect of the procedure itself upon a normal animal.

Dog 36 received several injections of small amounts of the Harris product with results as indicated in the protocol given below.

*Extract from protocol of dog 36,—39th day.* Dog refused food. Had convulsions involving chiefly the neck muscles and fore limbs at frequent intervals. At 11 a.m. dog was given 1 gram of Harris vitamine lot 965 by intraperitoneal injection. During the course of several hours convulsions occurred each, when appearing, usually beginning at the end of a deep inspiration. Slight improvement noticed during the afternoon. At 7 p.m. 2 grams of the same preparation were given intraperitoneally.

*40th day.* At 9 a.m. dog's condition appeared improved over that of the previous day. Dog drank much water; was able to rise to its feet without assistance, a feat which was not possible on the previous day. Food was refused. 5. p.m., great improvement noticed. At 5:45 p.m., dog was given 3 grams of the same vitamine preparation intraperitoneally.

*41st day.* At 9 a.m. dog was able to rise to its feet without assistance; had passed very dark colored feces during the night. Head swayed somewhat.

*42nd day.* 9 a.m., dog was much better, being able to walk about the room with a slightly incoördinated gait. Dog was very dirty, not being a fastidious animal; therefore given a warm bath.

Inasmuch as this experiment indicated that vitamin-B is absorbed when placed in the peritoneal cavity, but that when so administered in the small amounts tried with dog 36, brings about no really striking cure, it was decided to experiment using larger amounts of the Harris powder.

Dog 34 was a young male Dachshund which ate all the food offered for forty-three days; after three days of irregular food intake, it refused the food absolutely. At this time a muscular weakness developed, the animal being unable to support itself on its legs. The muscles of the neck were contracted, particularly those on the right side, resulting in the neck being twisted with the head toward the right. There were occasional convulsions during which the body assumed an opisthotonic position. At 2:45 p.m. 10 grams of the Harris vitamine were injected intraperitoneally. Two hours later the animal was placed on its feet whereupon it walked about the room, its gait exhibiting a moderate degree of incoördination. During the next half-hour this dog rose to its feet of its own accord several times and walked about the room. The great prostration, which was such a marked feature of the animal's condition at the time of the injection, had entirely disappeared. However, at midnight—nine and one-quarter hours after the injection—the animal was found dead. An autopsy revealed the presence of a large amount of bloody fluid in the abdominal cavity. No lesions of the intestine or other abdominal organs were found which might indicate that death was due to an internal hemorrhage caused by the injection needle injuring some blood vessel.

In this experiment the effect of the vitamin-containing preparation was unmistakable, the prostrated and convulsive animal being able to walk freely only two hours after receiving the injection. The fatal outcome nine hours later, however, suggested that the injection of 10 grams of the vitamine powder into the peritoneal cavity was too severe an experimental procedure. It was decided, therefore, to determine the effect of injecting only 5 grams of the Harris material into an animal suffering from vitamin-B deficiency.

Dog 35, which exhibited convulsions at frequent intervals and was unable to stand on its legs, received 5 grams of the Harris vitamine by the intraperitoneal route. During the first half-hour after the injection, convulsions still occurred; one hour after the treatment the animal still showed spasms which, however, were distinctly less pronounced. When three hours had elapsed since receiving the vitamin, all spasms had disappeared but the limbs were extended and their muscles were tense. Ten and one-half hours after the injection the dog was found dead. The autopsy findings were similar to those obtained with dog 34, a large amount of bloody fluid in the peritoneal cavity being the outstanding observation of interest.

A control experiment was now performed. A normal animal, dog 43, was given an intraperitoneal injection of 5 grams of the Harris vitamine powder. This dog, which had been unusually active, became very quiet as a result of this treatment and remained so for three days.

Inasmuch as this animal had been noisy and very difficult to handle and it still maintained its vicious behavior even after receiving the vitamin, no efforts were made to determine the effect of the injection upon the pulse rate, temperature, etc., the animal being undisturbed. For three days, however, the dog's behavior indicated that the effects of the injection had not disappeared. Five days elapsed before the animal regained its former active condition.

In the light of these experiments there seems to be no doubt that vitamin-B is absorbed readily when introduced into the peritoneal cavity and, when so given in a sufficient amount, brings about a rapid alleviation of the nervous and muscular symptoms characteristic of vitamin-B deficiency in the dog; however, the concentrated form of vitamin-B represented by the Harris product is not suited for intraperitoneal administration of vitamin-B.

*Subcutaneous injection.* Four dogs (nos. 46, 47, 48 and 49) were used for these experiments. Only one of these animals, dog 46, was brought through what might be called the *crisis* due to lack of vitamin-B as a result of subcutaneous administration of the Harris product. In this case treatment was begun before convlusions set in; the symptoms manifested were chiefly an incoördinated gait and a stiffness of neck muscles resulting in the animal walking with the head lowered. The improvement in the condition of the animal was slow, the stiffness of the neck musculature still being evident after twenty-four hours. Two other subcutaneous injections made on successive days resulted in a gradual disappearance of all symptoms and a return of the appetite, the latter not being completely restored, however, until four days after the last treatment was received.

With dogs 47 and 49, both of which exhibited severe convulsions similar to those which were treated successfully by intravenous injection of Harris vitamine, subcutaneous administrations of this product were without effect, dog 49 being found dead twenty-two hours later, and dog 47, its condition having grown progressively worse, being etherized and then bled and formalin injected into the circulatory system.[2]

Dog 48 did not show any of the severe nervous and muscular symptoms characteristic of vitamin-B deficiency; a loss of appetite was the

[2] In view of the differences of opinion that have developed regarding the histological pictures furnished by the tissues particularly the brain and spinal cord of beriberi patients and of animals suffering from vitamin-B deficiency, rapid fixation of all tissues *in situ* was adopted as a control procedure against possible post-mortem changes that may account for the different observations that have been reported in the literature. A study of the pathological changes in the tissues of such dogs as are described in this paper remains to be made.

only sign that the absence of the vitamin from the dietary had affected the animal. This dog was given a subcutaneous injection of the Harris product in order to determine whether the desire to eat might be restored by such a procedure. The loss of appetite was not influenced by this treatment although the lethargic condition of the animal was distinctly counteracted. Twenty days later the same animal was given the same amount of the Harris powder—neutralized and centrifuged—by stomach tube and as a result the appetite was restored completely over a period of four days. Administration of the same amount of the *unneutralized* material resulted in a similar restoration of appetite lasting for six days.

From these experiments it may be concluded that vitamin-B is absorbed when administered by the subcutaneous route; this absorption is so slow, however, that such a method of treatment is of no therapeutic value when treating cases of vitamin-B deficiency which have advanced to a point where convulsions occur; where the symptoms indicate the case to be only moderately advanced, a slow recovery may be brought about by several subcutaneous injections made on successive days. In view of the writer's experience, such cases may be treated more advantageously by giving the vitamin-containing product by mouth.

Discussion. The few experiments just described give added support to the idea that *polyneuritis gallinarum* and the peculiar syndrome induced in the dog by the absence of vitamin-B from the dietary are pathological conditions belonging to the same category.

These experiments also indicate—as do experiments on pigeons—that substances containing vitamin-B do not require action upon them by the enzymes contained in the various digestive juices preliminary to absorption before the vitamin contained therein can be effective in satisfying the organism's need for it. Inasmuch as the experiments here reported were made with only one source of vitamin-B, this conclusion cannot be accepted at once without question: further experiments with other sources must be performed, and an investigation made of the possibility that vitamin-B as it exists in foods, when introduced parenterally, is eliminated into the intestine, there to be acted upon in some way and then reabsorbed in an active form. The rapidity with which the parenterally introduced vitamin effects a relief from convulsions, however, would seem to argue against an excretion into the gut and a reabsorption therefrom.

In an earlier paper (2) were presented the results of a comparison of several extracts containing vitamin-B—rice polishings, wheat embryo, yeast, navy bean and tomato—for their appetite-promoting power in dogs and their ability to increase the flow of saliva, pancreatic juice

and bile. The effects of these extracts upon the arterial blood pressure when injected intravenously were not the same, in spite of the fact that all of these preparations contained considerable amounts of vitamin-B as shown by their great potency in restoring the appetite to dogs, which had lost the desire to eat after subsisting on a diet lacking this essential, and in curing polyneuritic pigeons. Therefore, experiments to determine the effect of parenteral administration of vitamin-B upon respiration, blood pressure and similar general properties of living organisms do not appear to possess any value at the present time; such studies must await the definite isolation of this vitamin as a chemical entity, an accomplishment which will then permit a systematic investigation of its pharmacological properties to be made.

## SUMMARY

A product rich in its vitamin-B content but free from protein—*Yeast Vitamine Powder (Harris)*—was tested for its effect upon the pathological symptoms exhibited by dogs, which had been fed a diet adequate except with respect to vitamin-B, the preparation being given to the animals by intravenous, intraperitoneal and subcutaneous injection.

In two out of three cases, intravenous administration of this product resulted in striking cures being brought about, severe convulsions being relieved within a half hour in one experiment, and within four and one quarter hours in the other. The third trial was inconclusive, the animal dying fifteen minutes after the injection was finished, death being due probably to heart failure, which often occurs among such animals.

Intraperitoneal injection of the Harris vitamine, when repeated at frequent intervals using doses of 1, 2 and 3 grams, resulted in a complete recovery from all nervous and muscular symptoms by the third day after the last injection in the single experiment of this character that was made. When a single large dose of 10 grams was thus injected into one animal, and 5 grams into another, striking relief from the spasms was obtained within two and three hours respectively, but death ensued in each case about ten hours after the treatment. A control intraperitoneal injection of 5 grams made into a normal dog gave definite indications that this procedure was in itself severe. It was concluded that the Harris vitamine is not suited for injection in large doses into the peritoneal cavity.

Treatments by subcutaneous injection of the Harris product into two avitaminotic dogs, which exhibited severe convulsions, were unsuccessful; in the case of a third animal the symptoms of which

indicated its condition to be unadvanced and of only moderate severity, a slow recovery was brought about by three daily subcutaneous administrations of this material.

## CONCLUSION

Dogs, like pigeons, may be relieved of their nervous and muscular symptoms characteristic of vitamin-B deficiency within the course of a few hours by intravenous or intraperitoneal injection of a sufficient amount of vitamin-B. This vitamin, when injected subcutaneously, however, is too slowly absorbed to be effective in bringing about such relief in severe cases; where the animal's condition is not critical, subcutaneous injections of vitamin-B have a therapeutic value which, however, is not superior to that of administration *per os*.

ACKNOWLEDGMENTS. The writer wishes to thank Dr. A. Chanutin, Dr. H. J. Deuel, Jr., and Messers Sidney Bliss and C. F. Failey, for assistance rendered at different times; he also wishes to express his appreciation of the services of Dr. R. R. Read, who took photographs of many of the animals.

The expenses of this investigation were defrayed in part by a contribution from the Russell H. Chittenden Research Fund for Physiological Chemistry.

## BIBLIOGRAPHY

(1) COWGILL: This Journal, 1921, lvii, 420.
(2) COWGILL AND MENDEL: This Journal, 1921, lviii, 131.
(3) DE LACERTA: Berl. klin. Wochenschr., 1886, xxiii, 159.
(4) GIBSON AND CONCEPCION: Philip. Journ. Sci., 1916, xi, 119.
(5) GRIJNS: Mededeelingen v.h. Lab. Path. Anat. en Bakt. te Weltevreden 1900. Cited by VEDDER.
(6) GRIJNS: Geneesk. Tydschr. v. Ned. Ind., xli. 1901. Cited by VEDDER.
(7) GRIJNS: Ibid., 1909, xlix, 216. Cited by GIBSON AND CONCEPCION.
(8) HOSE: Brit. Med. Journ., 1905, ii, 1098.
(9) KARR: Journ. Biol. Chem., 1920, xliv, 255.
(10) LUMIÈRE: Bull. Acad. Med. Paris, 1920, lxxxiii, 310.
(11) MCCARRISON: Studies in deficiency disease, Oxford Medical Publications, London, 1921.
(12) MOORE: Canada Exper. Farms Reports (1912), 1914, xiii, 611.
(13) MOORE: Canada Dept. Agric. Rpt. Vet. Dir. Gen. [1913], 1915, xiv, 137.
(14) OSBORNE AND WAKEMAN: Journ. Biol. Chem., 1919, xl, 383.
(15) ROMMELL AND VEDDER: Journ. Agric. Research, 1915, vl, 489.
(16) SCHAUMANN: Beih. z. Arch. f. Schiffs- und Tropenhyg., 1910, xiv, 325. Reviewed by VEDDER, chapter xi.
(17) SHIGA AND KUSAMA: Beih. z. Arch. f. Schiffs- und Tropenhyg., 1911, xv, 59. Reviewed by VEDDER, chapter xi.
(18) SUZUKI, SHIMAMURA AND ODAKE: Biochem. Zeitschr., 1912, xliii, 89.
(19) VEDDER: Beriberi. New York, 1913.
(20) VOEGTLIN AND LAKE: This Journal, 1919, xlvii, 558.
(21) WILLIAMS AND SEIDELL: Journ. Biol. Chem., 1916, xxvi, 431.

# THE EFFECT OF FATIGUED MUSCLE UPON PERFUSED ADRENALIN

R. G. HOSKINS AND E. P. DURRANT

*From the Laboratories of Physiology, Ohio State University*

Received for publication June 13, 1923

The studies of Cannon and Nice (1) and of Gruber (2) in recent years have shown that adrenalin has a remarkable energizing effect on skeletal muscle. This is shown more particularly in the rapid improvement of the action of a fatigued muscle, but according to Gruber's latest work (3), the non-fatigued muscle is likewise to some extent favorably affected. The literature on the subject, having recently been considered by Gruber (2), (3), need not be further discussed herewith.

This work raises the interesting question, how adrenalin produces such an effect. Considering nothing but the one phenomenon, one would assume as the most plausible explanation that the fatigue metabolites are neutralized. An alternative theory is, of course, merely that fuel oxidation is facilitated. Believing that the first mentioned mechanism might be involved, it seemed to us desirable to carry out a series of studies on the relation of adrenalin to muscle metabolism, with a view to determining, if possible, the chemical reactions involved It was decided first, however, to determine whether the adrenalin actually entered into any chemical combination in the muscle.

Accordingly, the experiments herein recorded were performed. So far as we are aware, only once before has the experiment been made of perfusing adrenalin through fatigued and through normal muscle and comparing the results. Carnot and Josserand (4) reported that 0.05 mgm. per kilo body weight injected into the femoral artery with the leg at rest produced a rise of blood pressure of 100 mm. When, after tetanizing the opposite leg for fifteen minutes, 0.055 mgm. of adrenalin per kilo body weight was injected into its femoral artery, the blood pressure now rose only 15 mm.

In our experiments the animals were anesthetized with ether administered by tracheal tube. Variations in depth of anesthesia as significant factors in the results were, of course, excluded. Both vagi were cut with a view to stabilizing blood pressure. The right sciatic nerve was exposed and cut near the head of the femur and to the peripheral end a Sherrington electrode was attached. In some cases the femoral nerve was also

cut, but this procedure apparently made no difference in the results. The abdomen was opened, exposing the juncture of the common iliac arteries. The left artery was cannulated and served as a portal of entry for the adrenalin solution to the right iliac artery. The median sacral artery and any smaller arteries near the iliac juncture were ligated. During the progress of these experiments a technic very similar to this was described by Ranson (5). The cannula was connected with a buret and both were filled with adrenalin solution at a concentration of 1/100,000 to 1/500,000. The buret was connected with a pressure bottle through a T-tube connected with a mercury manometer. The pressure in this system was kept approximately uniform throughout the experiment, at about 200 mm. of mercury.

In some cases the tibialis anticus muscle was connected to a writing lever, and attached to pull against a calibrated spring. This was arbitrarily set in case of each animal so as to give a registered graph of convenient size. In other cases the graphs of muscle contraction were omitted, the leg being tetanized to a point of fatigue as shown by resulting flaccidity of the muscle. Arterial blood pressure was registered from the carotid. Calibrating doses of adrenalin were first injected under constant pressure and at a constant rate. The muscle was then fatigued and the same doses again injected. The systemic reactions were taken to indicate whether any more adrenalin had been absorbed in its passage through the fatigued muscles than through the normal. Since fatigued muscle reacts more extensively to perfused adrenalin than does non-fatigued, it was assumed that if any chemical reaction between the drug and the muscle were involved the systemic reaction would be lessened after fatigue. Even without this assumption our data indicate that little or no adrenalin was removed from the blood during its flow through the muscle because the systemic reactions to the drug administered by artery were very similar to those obtainable when it is given by vein.

In all, seven cats and twenty-two dogs were used. In case of cats the dosage varied from 0.0002 mgm. to 0.02 mgm. per kilogram body weight. In case of the dogs the dosage ranged from 0.0004 mgm. to 0.001 mgm. per kilo. It was found that to demonstrate the accentuating effect of adrenalin on fatigued muscle contractions dosages of 0.002 to 0.006 were usually satisfactory.

The results may be briefly stated. Whether pressor or depressor doses of adrenalin were used there was no significant difference in the systemic reaction, whether the adrenalin was perfused through non-fatigued or fatigued muscle. In some cases the effect was slightly greater and in some it was slightly less, but these variations were no greater than one sees in the reactions to successive doses when administered under ordinary conditions by vein. It is evident, therefore, that

the energizing effect of adrenalin does not depend upon any chemical reaction into which it enters. The effect must, therefore, be due to catalysis.

That this is true of muscle metabolism could have been deduced with some degree of probability from the fact that adrenalin produces a sharp increase in the basal metabolic rate even when administered in minute quantities. For example, Boothby and Sandiford (6) have detected a significant increase when as little as 0.2 mgm. was administered subcutaneously to an adult human patient. Absorption under such conditions is relatively slow. Obviously, effects of this magnitude from such small quantities are to be ascribed to catalysis.

As was shown by Hoskins, Gunning and Berry (7), adrenalin in such dilutions as were used produces vasodilatation in the blood vessels of muscles. Gruber (2), however, found that this effect is not demon-

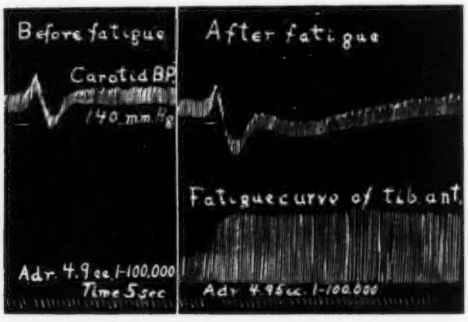



Fig. 1

strable when muscle has been newly denervated, hence local circulatory reactions presumably played no part in the results of these experiments.

The fact that adrenalin remarkably increases heat production and our observations that the drug does not itself enter into the chemical reactions involved in muscle metabolism, obviously suggest a possible normal function of the adrenal glands, namely, to supply a respiratory catalyst. This theory has, of course, been familiar to physiologists for many years. Sajous (8) in particular has emphasized it. The work of Stewart and Rogoff (9), showing that cats with denervated adrenals and discharging no detectable amount of adrenalin remained in excellent health, serves to preclude any theory of adrenal function based on the ordinary pharmacologic reactions to this substance except under short lasting periods such as are assumed in Cannon's emergency theory. Boothby and Sandiford's results, previously quoted (6), however, suc-

cessfully escape the quantitative objection. That is to say, adrenalin has been shown to produce marked effects in concentrations below the limits detectable by ordinary tissue reactions. The fact that some animals survive in good health after adrenal denervation is paralleled by the fact that a denervated thyroid graft is sufficient to prevent symptoms of hypothyroidism. This observation indicates that an incretory gland, even though it may receive stimulatory fibers, is able to function effectively without them. It is to be noted, however, that the cats in which Hartman (10) denervated one adrenal and removed the other experienced a period of ill health.

## SUMMARY

1. In seven cats and twenty-two dogs the comparative effects of fixed quantities of adrenalin were determined when perfused through normal muscle and fatigued muscle.

2. The fact that adrenalin materially increases the activity of skeletal muscle was readily confirmed.

3. No significant difference in the systemic effects of the adrenalin could be detected whether perfused through a normal or a fatigued muscle.

4. This fact indicates that adrenalin acts merely as a catalyst.

5. This fact in connection with observations of other investigators on the relation between adrenalin and basal metabolism indicates as a possible function of the adrenal glands the incretion of a respiratory catalyst.

It is a pleasure to acknowledge our indebtedness to Mr. R. R. Durant whose skilful aid played a large part in the successful conclusion of these experiments.

## BIBLIOGRAPHY

(1) Cannon and Nice: This Journal, 1913, xxxii, 44.
(2) Gruber: Endocrinol., 1919, iii, 145.
(3) Gruber: This Journal, 1922, lxii, 438.
(4) Carnot and Josserand: Compt. rend. Soc. de biol., 1902, liv, 1472; cit. from Gruber (2).
(5) Ranson, Faubion and Ross: This Journal, 1923, lxiv, 311.
(6) Boothby and Sandiford: Personal communication by Dr. Boothby.
(7) Hoskins, Gunning and Berry: This Journal, 1916, xli, 513.
(8) Sajous: Endocrinol., 1917, i, 4.
(9) Stewart and Rogoff: Journ. Pharm. Exper. Therap., 1917, x,1.
(10) Hartman, Waite and Powell: This Journal, 1922, lx, 255.

# STUDIES ON THE LOCOMOTION OF LEUCOCYTES

## I. The Normal Rate of Locomotion of Human Neutrophilic Leucocytes in Vitro

MORTON McCUTCHEON

*From the McManes Laboratory of Pathology, University of Pennsylvania Medical School*

Received for publication June 5, 1923

In contrast to the numerous and careful quantitative studies on phagocytosis by leucocytes, very little attention has been paid to the rate of locomotion of these cells. There is abundant evidence that leucocytes behave very differently in various conditions of health and disease, and it is likely that their rate of locomotion is influenced by factors which are at present imperfectly understood. The present quantitative studies of motility were undertaken with the hope of gaining information about some of these factors.

It seemed desirable in the first place to determine the rate of locomotion of normal human cells in vitro. The only data available on this subject are given by Jolly (1) and Comandon (2). These workers took moving pictures of leucocytes creeping about between slide and coverslip. These pictures were projected and drawings made of the outline of the cell at known time intervals. By measuring the distance which a cell had moved, the rate of locomotion could readily be calculated.

The figures reported by Jolly and Comandon, respectively, are rather widely different. And since it was found as the result of preliminary experiments that slight changes in technic have a marked effect on the values obtained, it was deemed necessary to develop a standard technic, by which the normal rate of locomotion might be determined, to serve as a basis for future experiments.

*Technic.*[1] The slides and coverslips must be most *scrupulously*

[1] In a series of preliminary experiments, it was sought to develop a technic whereby exudative cells might be studied in physiological solutions. These experiments were only partially successful, inasmuch as the results were inconstant, and the cells suffered early injury and disintegration. Dr. Florence R. Sabin, of Johns Hopkins Medical School, then very kindly demonstrated her technic to the author, which consists in using a single drop of undiluted blood. This method gave such dependable results that it was adopted by the author, who desires to express his indebtedness to Doctor Sabin both for her suggestions and encouragement.

clean,[2] as a speck of dust or trace of grease tends to reduce the velocity obtained. A drop of blood is obtained by puncture from the finger in the usual manner, and the center of a coverslip is touched to the drop. The coverslip is then placed on a slide, whereupon the drop of blood spreads out uniformly. In case an even spread is not obtained, the preparation should be rejected.

The coverslip is then ringed with vaseline having a high melting point, and placed on the stage of the microscope in a warm box.[3] The bulb of a thermometer rests on the stage of the microscope, and the temperature is maintained at 37 $\pm$ $\frac{1}{2}$ °C. Thirty minutes or more are allowed for the cells to recover from the effects of chilling and manipulation. The cells are then examined with a Leitz 6 millimeter objective and a Leitz drawing ocular, by means of which the image of the cell is projected onto a piece of paper and its center marked on the paper by a dot. Observations are taken at half-minute intervals for five minutes. A series of dots is thus obtained, indicating the course of the leucocyte as it moves across the field. The distance between consecutive dots is then measured with a millimeter rule, and the ten values thus obtained are added, giving the approximate distance travelled by the cell in five minutes. After determining the magnification by means of a stage micrometer, the distance travelled in five minutes is expressed in micra. In this way ten cells are successively examined in each experiment. The results given below are based on ten such experiments carried out within five days on the blood of one individual, a healthy man.

The length of time for which a preparation may be kept without deterioration has not been determined, but after three hours no sensible diminution in the velocity of the cells can be observed. This finding is at sharp variance with the statement of Comandon (2) that at 37° deterioration begins in a few minutes.[4] There seems no doubt, however, that such change does not occur within several hours if the technic described above is adhered to; and data in support of this statement will be given in a subsequent paper.

Only the finely granular (neutrophilic) leucocytes were studied. No difficulty is found in recognizing these with the magnification used and without supra-vital staining. Eosinophiles are readily distinguished by their large refractile granules. Monocytes are hyaline, without obvious

[2] Slides and coverslips were placed one by one in acid alcohol over night, washed in running water, placed in alcohol, then ether. Coverslips were polished with a silk handkerchief. Slides were rubbed with jewelers' rouge and then polished with a silk handkerchief.

[3] For description of warm box, see second article of this series appearing in present number of this Journal.

[4] Unfortunately Comandon did not give his technic.

granules, and do not show streaming of the cytoplasm. Lymphocytes can be told by their small size and relative immobility. In making these observations, leucocytes not showing definite locomotion were disregarded.

*Results.* In table 1 is tabulated the distance travelled by each of 100 finely granular leucocytes in five minutes. This distance is found to range from 90 to 237 micra, with an average of 170.6 ± 2.2 in five minutes, or 34.1 micra per minute.

TABLE 1

*The distance in micra travelled by each of 100 cells in five minutes*

| | NUMBER OF EXPERIMENT | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Cell 1..... | 120.0 | 150.0 | 137.5 | 170.0 | 175.0 | 175.0 | 150.0 | 175.0 | 187.5 | 180.0 |
| Cell 2..... | 135.0 | 165.0 | 127.5 | 145.0 | 157.0 | 197.5 | 197.5 | 145.0 | 195.0 | 150.0 |
| Cell 3..... | 110.0 | 152.5 | 177.5 | 130.0 | 170.0 | 132.5 | 215.0 | 150.0 | 172.5 | 142.5 |
| Cell 4..... | 185.0 | 180.0 | 167.5 | 90.0 | 167.5 | 157.5 | 237.5 | 210.0 | 145.0 | 225.0 |
| Cell 5..... | 180.0 | 172.5 | 172.5 | 127.5 | 202.5 | 137.5 | 215.0 | 170.0 | 137.5 | 220.0 |
| Cell 6..... | 107.5 | 190.0 | 162.5 | 197.5 | 142.5 | 105.0 | 177.5 | 185.0 | 177.5 | 207.5 |
| Cell 7..... | 177.5 | 222.5 | 150.0 | 200.0 | 235.0 | 162.5 | 117.5 | 170.0 | 162.5 | 222.5 |
| Cell 8..... | 222.5 | 167.5 | 152.5 | 132.5 | 205.0 | 175.0 | 180.0 | 185.0 | 215.0 | 130.0 |
| Cell 9..... | 120.0 | 145.0 | 147.5 | 170.0 | 197.5 | 220.0 | 185.0 | 192.5 | 197.5 | 182.5 |
| Cell 10..... | 172.5 | 217.5 | 120.0 | 197.5 | 130.0 | 110.0 | 237.5 | 195.0 | 170.0 | 227.5 |
| Totals..... | 1530.0 | 1762.5 | 1515.0 | 1560.0 | 1782.5 | 1572.5 | 1912.5 | 1777.5 | 1760.0 | 1887.5 |

Table 1 also shows that the individual cells differ in their velocity within rather wide limits, and that this is true even of cells in the same preparation. However by means of a graph (fig. 1) it may be seen that the velocities of most of the cells lie near the arithmetic mean. I have plotted the frequency distribution of the observations grouped in units of 20 micra. The resulting graph approximately conforms to a normal probability curve; it has a mean value of 170.0 micra and a standard deviation of 33.3 micra.

The rate of locomotion reported by Jolly for human leucocytes is 15.5 micra per minute at 37°—a velocity less than half that which was found in the present series. But the figures given by Comandon—an average of 25.2 micra per minute at 35°—agree quite closely with mine, provided that allowance be made for the difference in temperature. The effect of temperature on locomotion will be discussed in a subsequent paper (3). For the present it may be stated that, within certain limits, an increase in temperature of 10° approximately doubles the velocity. From this relation it may be calculated that if a cell moves at the rate

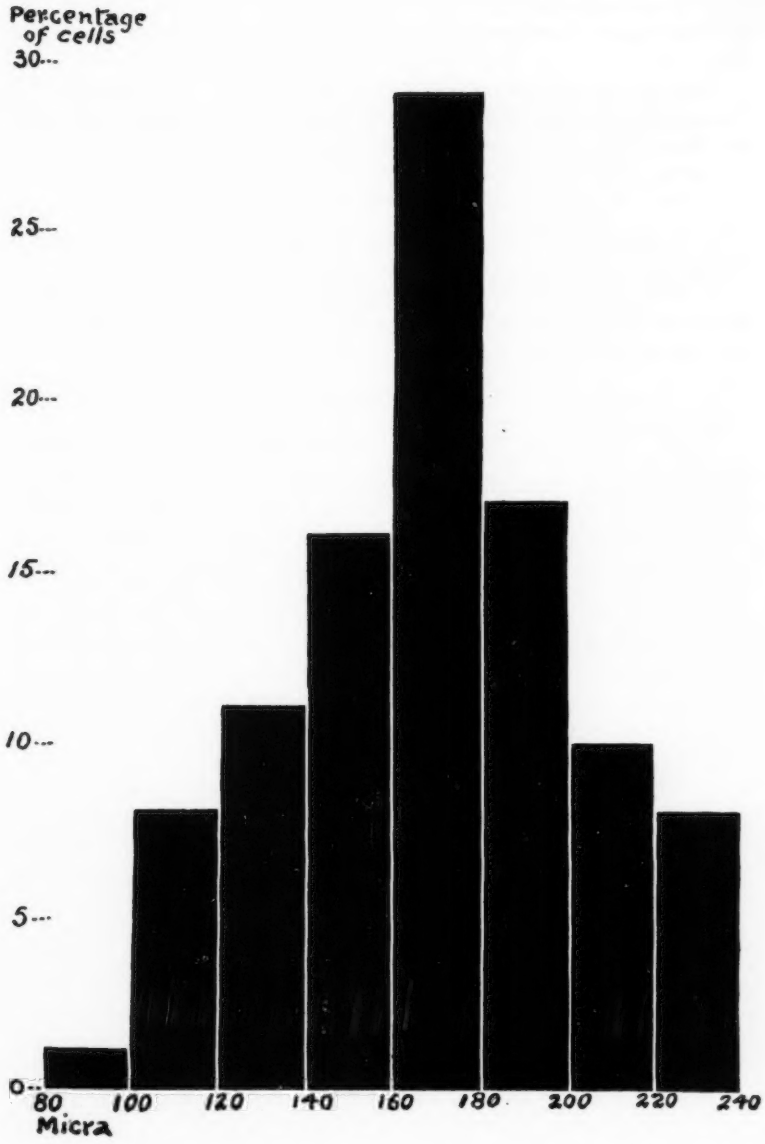



Fig. 1. Frequency distribution of 100 leucocytes, grouped according to distance travelled in 5 minutes.

of 25.2 micra per minute at 35° (as found by Comandon), then at 37° it would have a velocity of about 28.9, as against my mean velocity of 34.1.

There is a notable agreement also in the maximal values obtained by Comandon and myself, Comandon finding 46.8 micra (with correction for temperature, 53.7) as against my maximum of 47.4.

## SUMMARY

1. A method is given for determining the velocity of normal human neutrophilic leucocytes examined in whole blood in vitro.

2. In the case of the individual chosen the average rate of locomotion, under the conditions described, was 170.6 ± 2.2 micra in 5 minutes, or 34.1 micra per minute, at 37°C.

## BIBLIOGRAPHY

(1) JOLLY: Compt. rend. Soc. d. Biol., 1913, lxxiv, 504.
(2) COMANDON: Ibid., 1919, lxxxii, 1305.
(3) McCUTCHEON: This Journal, 1923, lxvi, 185.

# STUDIES ON THE LOCOMOTION OF LEUCOCYTES

## II. The Effect of Temperature on the Rate of Locomotion of Human Neutrophilic Leucocytes in Vitro.

MORTON McCUTCHEON

*From the McManes Laboratory of Pathology, University of Pennsylvania Medical School*

Received for publication June 5, 1923

In studying the velocity of any biological process it is of great importance to recognize the influence that is exerted by the temperature at which the observation is made.

The well-known law of Van't Hoff states that the velocity of a chemical reaction is approximately doubled or trebled by a rise in temperature of 10°C. Now, it is of great interest that the same law has been found to hold for various biological phenomena. To mention only a few examples, Kanitz (1) studied the frequency of contraction in the isolated heart of the rabbit and dog; Snyder investigated such phenomena as the rate of transmission of nerve impulses in the pike and frog (2), and the rhythmic contraction of mammalian small intestine (3); in all these cases, the velocity was found to be increased two to three times by a rise in temperature of 10°. Eberling (4) has recently found that wounds in the skin of alligators heal twice as rapidly when the temperature increases 10°. A curious application of this rule has been described by Strumper (5) in the case of ants. He reports that these insects move twice as rapidly at 21° as at 11°, and that the number of combats resulting from encounters between individuals is nearly twice as great at the higher temperature.

The factor by which a velocity at a given temperature must be multiplied to find a theoretical velocity at a temperature 10° higher is commonly written as $Q_{10}$. The value of this temperature coefficient is conveniently found by the formula employed by Kanitz (1) and Fenn (6), $\log Q_{10} = \frac{\log K_1 - \log K_2}{t_1 - t_2} \times 10$, in which $K_1$ is the velocity at the higher, $K_2$ at the lower temperature, and $t_1 - t_2$ is the difference in the temperatures at which the two observations are made.

In the case of the rate of locomotion of human leucocytes, the somewhat scanty data of Comandon (7) have recently been worked up by

Fenn (6), who found that, comparing the velocities at 25° and 30°, $Q_{10} = 4.6$, while between 30° and 35° $Q_{10} = 1.5$.

It seemed, however, desirable to obtain more extensive data on this subject, and to determine with what degree of regularity the rate of locomotion of neutrophilic leucocytes was affected by changes in temperature over a wide range.

Now, in any reaction it is evident that the law of Van't Hoff will apply only over a certain range of temperature changes. In biological processes, too great an elevation of temperature will certainly damage and ultimately kill the cells, so that at the upper end of the range, the temperature coefficient would fall. Again, at low temperatures there is a disproportionate slowing of the process. Thus Van't Hoff (quoted by Kanitz) (1) found that at low temperatures the value of $Q_{10}$ might be as high as 5 or 6. In the phagocytosis of carbon particles by leucocytes, Fenn (6) found that above 30°, $Q_{10}$ is equal to 1.41 but below 30°, the high value of 18.6 is obtained.

*Experimental.* The technic used in determining the rate of locomotion of human neutrophilic polymorphonuclear leucocytes was described in the first paper of this series (8). A drop of whole blood is mounted between slide and coverslip and examined under the microscope which stands in a warm box. The warm box used in the present experiments was modelled after those employed by Dr. W. H. Lewis and Dr. F. R. Sabin of Johns Hopkins University Medical School. Certain changes were made to allow the tube to be tilted at 45°, as is necessary for the use of a drawing ocular. The box is made of wood, and is 40 cm. in width, 34 in depth and 28 in height. The tube of the microscope passes through an opening in the front. The sides are provided with sliding doors, to permit entrance to the hands. Heat is obtained from three electric lights which may be turned on or off as required. The top is in two pieces, and is not fastened on; by separating these two pieces, warm air is allowed to escape from the interior, and in this way the temperature may be reduced. A thermometer lies horizontally in the box with its bulb resting on the glass slide on which the preparation is mounted; frequent readings are made through a glass window in the front of the box, and the temperature is not allowed to fluctuate more than half a degree plus or minus.

A drawing ocular is used for measuring the distance travelled by a cell in a given time.

The simplest way to compare the rates of locomotion at different temperatures would have been to study one or more preparations at a certain temperature, and then subsequent preparations at various other temperatures, comparing then the mean velocities obtained. But, as may be seen from table 1, there is some variation in the rate of locomo-

tion in different preparations, due probably to very slight differences in the thickness of the spreads. Therefore the following procedure was adopted. The preparation was mounted and allowed to remain for 30 minutes at 37° to give time for the cells to become adapted to conditions in vitro and to recover from chilling. Then the distance travelled by a cell in five minutes was measured as previously described (8), and afterwards other cells were chosen until 10 had been examined; usually it was possible to work with 2 to 4 cells simultaneously. After this was done, the temperature was raised or lowered to another mark, for example 33°, and after again allowing half an hour to elapse for readaptation of the cells, the distance travelled by 10 cells at this temperature was measured. The leucocytes studied during the two observations might be the same or different ones.

TABLE 1

*The rate of locomotion of leucocytes at different temperatures. In each experiment is recorded the average distance travelled by 10 cells in 5 minutes. In the series of experiments recorded in the left hand side of the table the observations were made, first at 37°, and then at some other temperature, using the same preparation, while in the series recorded in the right hand side, this order was reversed.*

| EXPERIMENT NUMBER | TEMPERATURE | MEAN DISTANCE TRAVELLED IN 5 MINUTES | TEMPERATURE | MEAN DISTANCE TRAVELLED IN 5 MINUTES | EXPERIMENT NUMBER | TEMPERATURE | MEAN DISTANCE TRAVELLED IN 5 MINUTES | TEMPERATURE | MEAN DISTANCE TRAVELLED IN 5 MINUTES |
|---|---|---|---|---|---|---|---|---|---|
| | °C. | *micra* | °C. | *micra* | | °C. | *micra* | °C. | *micra* |
| 1 | 37 | 185.0 | 23 | 31.4 | 2 | 23 | 54.2 | 37 | 187.0 |
| 3 | 37 | 166.4 | 27 | 82.8 | 4 | 27 | 90.6 | 37 | 181.9 |
| 5 | 37 | 200.0 | 31 | 135.3 | 6 | 31 | 131.1 | 37 | 190.4 |
| 7 | 37 | 177.2 | 33 | 134.6 | 8 | 33 | 128.9 | 37 | 175.0 |
| 9 | 37 | 166.4 | 40 | 194.9 | 10 | 40 | 199.7 | 37 | 153.9 |
| 11 | 37 | 180.1 | 42 | 153.7 | 12 | 42 | 153.0 | 37 | 166.7 |

It was thought possible, however, that the cells might deteriorate during the course of an experiment, so that too low a reading might be obtained during the second observation. To neutralize such an error, a second preparation was then made and the experiment done in the reverse order; first a determination was made at 33° and then one at 37°.

In two such experiments the distance travelled by 20 leucocytes at two different temperatures was measured. The mean velocities at 33° and 37° were then compared, and the value of $Q_{10}$ calculated as described above. Similar pairs of experiments were done at 23°, 27°, 31°, 40° and 42°, in each case comparing the velocity at the temperature selected with the velocity at 37°. In this way a series of values of $Q_{10}$ was obtained which are given in table 2 together with the mean velocity at each temperature.

The average rate of locomotion at 37° was found to be 177.5 micra in 5 minutes. The maximum rate was obtained at 40°, with a value of 197.3, and the minimal rate was at 23°, with 42.8 micra (see table 2). Wlassow and Sepp (9) also obtained the maximal locomotion of polynuclear leucocytes at 40°, while Comandon (7) thought that it was in the neighborhood of 38°.

The results are interesting in showing that Van't Hoff's law is found to hold strictly between the limits 27° and 40°, and that within these limits $Q_{10}$ is nearly constantly equal to 2. This means that between 27° and 40°, at least at the several temperatures employed, the rate of locomotion is doubled by an increase in temperature of 10°.

It will be observed, however, that at the lowest temperature investigated, 23°, the value of $Q_{10}$ becomes greater than 2, that is, that as the temperature is lowered below 27° there occurs a disproportionately great slowing of locomotion. This, as already stated, is found to be the case at low temperatures both in chemical reactions and in more complex biological processes. Fenn (6) has supposed that at low temperatures there is a rapid increase in the viscosity of the cell. Again at 42°, it will be observed that the value of $Q_{10}$ falls to 0.78, meaning that at 42° the velocity is actually slower than at 37°.

This same effect of high temperatures on leucocytes has already been observed by Wlassow and Sepp (9). My observations agree with those of these writers in indicating that several factors here are operative. One of these is an increased fluidity of the cell, which tends toward more rapid locomotion; and in fact some of the cells were found to travel with relatively great rapidity at 42°. But opposing this tendency to acceleration is an incoördination in the movements of the cells (better seen, however, at 45°); pseudopods are often sent out in opposite directions (this occurs to a less extent at normal temperatures) so that the cell becomes much attenuated, without, however, greatly changing its position in the field. Other cells, again, show simple depression of locomotion, and move coördinately but slowly. As a result there is a wide variation in the rate of locomotion, as indicated by the high value of the probable error of the mean found at 42° (see table 2). The net result is a retardation of locomotion.

The possibility of deterioration of the leucocytes during the course of the experiments was mentioned earlier in this paper. Study of the readings obtained seems to show that this fear was not justified. In table 1 are given velocities at 37° when the preparation was examined at this temperature first, and in the last column, when the cells were studied at 37° at the end of the experiment; that is, when deterioration might have occurred. It will be seen that all the figures are within normal limits, so that there is apparently no significant decrease in rate of locomotion during a 2 to $2\frac{1}{2}$ hour experiment.

This conclusion is in conflict with the findings of Comandon (7), who states that deterioration appeared within a few minutes at 37°.

It should be emphasized that all the experiments here reported were done on the blood of a single, supposedly healthy, adult man.

Discussion. From the results reported above, the conclusion is obvious that the results of locomotion experiments in vitro would be invalidated by failure to control the temperature rigidly.

Also there are certain generally recognized facts in regard to resistance to infection which are in harmony with these observations. Thus, many local infections are benefited by heating the part. Now, the

TABLE 2

*The average rate of locomotion of leucocytes at various temperatures, as compared with the rate of locomotion at 37° in the same preparations. This table represents a combination of the right and left hand sides of table 1, so that each value is based on observations on 20 leucocytes. In the last column are recorded the temperature coefficients obtained from the ratios of the velocity at 37° with the velocity at various other temperatures*

| TEMPERATURE | DISTANCE TRAVELLED IN 5 MINUTES | TEMPERATURE | DISTANCE TRAVELLED IN 5 MINUTES | $Q_{10}$ |
|---|---|---|---|---|
| °C. | *micra* | °C. | *micra* | |
| 23 | 42.8 ± 2.6 | 37 | 186.0 ± 4.4 | 2.86 |
| 27 | 86.7 ± 3.2 | 37 | 174.1 ± 5.0 | 2.01 |
| 31 | 133.2 ± 3.0 | 37 | 195.2 ± 4.2 | 1.89 |
| 33 | 131.8 ± 4.2 | 37 | 176.1 ± 2.8 | 2.06 |
| 40 | 197.3 ± 4.1 | 37 | 160.2 ± 6.9 | 2.00 |
| 42 | 153.3 ± 6.8 | 37 | 173.4 ± 4.3 | 0.78 |

factors that are modified by the application of heat are probably numerous and complex, but it is possible and even likely that one of these is the rate of locomotion of leucocytes into an infected area; for phagocytosis and liberation of enzymes are dependent on the prompt emigration and arrival of leucocytes at the site of infection.

Considering the temperature of the body as a whole, it is interesting to note that the most rapid rate of locomotion occurred at 40°, corresponding to a marked pyrexia, or temperature of 104°F., whereas there was a depression of locomotion at 42°, a temperature of hyperpyrexia, corresponding to a fever of 107.6° F. An individual with a fever of 104°F. is generally considered as showing a good reaction to infection, but one with a temperature of 107.6° is regarded as in an alarming condition, and steps are often taken to reduce such a fever by artificial means. The effect of such temperatures on the locomotion of leucocytes may be one reason why the prognosis is usually more favorable if the temperature does not exceed 104°. But that the same relation of locomotion of leucocytes to temperature holds in vivo as in vitro remains to be shown.

SUMMARY

1. The velocity of locomotion shown by neutrophilic polymorphonuclear leucocytes of one individual is found to be doubled, under the conditions stated, in vitro, by a rise in temperature of 10°, within certain limits of temperature variation.

2. This constitutes another instance in which van't Hoff's law is found to hold for biological phenomena.

BIBLIOGRAPHY

(1) KANITZ: Pflüger's Arch., 1907, cxviii, 601.
(2) SNYDER: Arch. f. Anat. u. Physiol., (physiological part), 1907, 113.
(3) SNYDER: Ibid., 126.
(4) EBERLING: Journ. Exper. Med., 1922, xxxv, 657.
(5) STRUMPER: Compt. rend. Soc. d. Biol., 1921, lxxxv, 706; Ibid., 708.
(6) FENN: Journ. Gen. Physiol., 1922, iv, 331.
(7) COMANDON: Compt. rend. Soc. d. Biol., 1919, lxxxii, 1305.
(8) McCUTCHEON: This Journal, 1923, lxvi.
(9) WLASSOW AND SEPP: Virchow's Arch., 1904, clxxvi 185.

# SOME EFFECTS OF VARIOUS ENVIRONMENTAL TEMPERATURES UPON THE BLOOD OF DOGS[1]

FREDERICK B. FLINN AND ERNEST L. SCOTT

*From the United States Public Health Service and the Department of Physiology, Columbia University, New York*

Received for publication June 13, 1923

Our object in undertaking this research was to acquire a more intimate knowledge of the specific effects of high environmental temperatures upon certain individual organs and tissues of the body, so that general effects as observed among furnace workers could be interpreted more accurately than has heretofore been possible.

Unfortunately, there are no animals in common use in the laboratory which are wholly suitable for such a study. Of the domestic animals the horse is perhaps to be preferred because of the greater similarity of its heat-controlling apparatus to that of man, but inasmuch as facilities for handling animals of this size were not available we were compelled to consider the smaller animals. Of these, dogs were selected as being the most tractable and, all things considered, the best adapted for the work which we planned to do. In making this selection it was frankly recognized that the dogs have developed a method of cooling the body by the evaporation of water which is strikingly different from that which obtains in the horse and man. However, in spite of this dissimilarity and its possible effects upon the gases of the blood, our results seem to indicate that any differences are quantitative and that the qualitative changes in gas content which result from exposure to high temperatures are the same for the two forms. Aside from this theoretical objection the dogs proved to be as nearly ideal as could be hoped for.

We have centered our attention on a single tissue—the blood. This tissue was chosen because of the rapidity and accuracy with which it reflects changes taking place throughout the organism, and because it is the only tissue which may be sampled at intervals and still leave the animal in what may be presumed to be an approximately normal condition. The blood was analyzed for oxygen content and capacity, carbon dioxide content and capacity, sugar and total solids,

[1] Approved for publication by the Surgeon General.

all of which were determined as a matter of routine. In a few cases the iron of the blood was determined as a control on the oxygen capacity. In a second series the hydrogen-ion concentration and the carbon dioxide content of the plasma were determined as routine, while an occasional determination of the lactic acid in the blood was made.

In presenting the data which we have accumulated, we are aware that various investigators, from the time of Claude Bernard on, have reported observations on the blood of animals which were exposed to high temperatures, but it seems to us that these results are not only fragmentary but in some cases of such doubtful accuracy that a clear-cut interpretation is practically impossible. The inaccuracies, where they exist, are due not only to faulty chemical technic but frequently to an abnormal condition of the animals. For example, many of them were subjected to anesthesia, which disturbs not only the heat-regulating mechanism but also the general metabolism of the subject. Furthermore, these observations were made at various times upon various species of animals, while in the present research we have endeavored to correlate as many data and data of as many different types as is practical on a given series of individuals of a single species.

In addition to the previously mentioned observations on the blood, we have recorded the rectal temperature and the body weight whenever a sample of blood was drawn, and in certain of the experiments even more frequently.

METHODS: I. *Chemical.* *a. The blood gases.* The carbon dioxide content and capacity of the blood were determined by the methods of Van Slyke (1). The oxygen content and capacity were determined by the method of Van Slyke and Stadie (2), but we found it necessary to add three or four drops more of the potassium ferricyanide solution than is recommended by these authors, possibly because our ferricyanide was the product of a different manufacturer.

All of the results reported were obtained with the old form of the Van Slyke apparatus, having the short stem. This apparatus was checked against the newer form having the longer stem and the water jacket and we did not feel that the differences in the results were sufficient to warrant the extra time required by the latter apparatus; for this would have necessitated an additional observer and, in our opinion, the differences due to personal equation would have more than offset the greater precision of the later apparatus.

*b. The sugar of the blood.* MacLean's method for 1 cc. of blood (3), as modified by Hastings and Hopping (4), was used throughout the work.

*c.* The total *iron* of the blood was occasionally determined as a means of controlling the oxygen determinations, using the method published by Brown (5). In this work we found that the colorimeter gave a more satisfactory means of comparing the colors than did the method described by the author.

*d.* The *lactic acid* was determined by the method which we have described elsewhere (6) except that the filtrate was extracted with ether and the determination made on the ether extract instead of on the filtrate directly. This was done upon the suggestion of Dr. Isidor Greenwald, in order to avoid the disturbing influence of the sugar of the blood. It is recognized that this method is not specific for lactic acid but since we were unable to find indications of an increase we feel that it is sufficient to justify the conclusions which we have drawn.

*f.* The *total solids* were determined by drying 1 cc. of blood to constant weight in an electric oven at 110°C. This was done in duplicate in silica crucibles.

*g.* The hydrogen-ion concentration was determined by the colorimetric method described by Cullen (7). The phosphate solutions which were used as standards of comparison were checked by means of the potentiometer.

II. *Physical. a. The temperature chamber.* The chamber in which the animals were exposed to the various environmental conditions was constructed of two layers of beaver board separated by a 4-inch air space. The chamber contained three windows, one at one end, another on one side and a third on the top. These windows were each about 2 feet square, and consisted of two sheets of glass with a 2-inch air space between them. A gas stove was placed inside at one end, under the window; at the opposite end there was a single wooden door lined with beaver board. The inside dimensions of the chamber were 4 feet in width by 7 feet each in length and height.

The method of heating, of heat control and of ventilation was that described by Hastings (8), and it has proven very satisfactory. The gas flow was controlled by a Roux regulator. A 6-inch electric fan was installed to overcome stratification and pockets in addition to the use of convection currents as recommended by Hastings. Thermometers placed in various parts of the chamber showed no stratification or variations in temperature greater than one-half of one degree Centigrade, while the temperature of the chamber as a whole did not vary more than one degree throughout the day.

*b.* The *relative humidity* within the chamber was determined by means of a sling psychrometer.

*c.* The *body temperature* was taken by an ordinary certified 1-minute clinical thermometer inserted in the rectum. The thermometer was left in place for at least $1\frac{1}{4}$ minutes.

*d.* The *respiratory rate* was counted by means of a Fitz pneumograph writing on a smoked drum through a Marey tambour. This technic was made necessary by the extremely high rates of respiration encountered at the higher temperatures.

III. *Biological.* All of the dogs used in these experiments were adult, short-haired mongrels, varying in weight from 10 to 15 kilograms. They were kept, when not actually in use for the experiments, in a kennel on the roof, and were maintained in a healthy condition throughout. It is needless to say that they were used exclusively for the purpose of the research here reported.

Their diet consisted of bread and cooked meat in an amount at least sufficient to maintain their weight. As a matter of fact, most of them gained in weight during the course of the experiments. They received no food during the period of 18 hours preceding each experiment. No food or water was permitted during the period of the experiments.

It was our practice to bring the dog which was to be used for an experiment to the laboratory at least half an hour before actually beginning the work, in order that he might become quiet and somewhat accustomed to conditions and to the personnel of the laboratory before the initial sample of blood was drawn. A rest period of at least two weeks was permitted each dog between experiments to allow recovery from any deleterious effects caused by the high temperature or from the hemorrhage attendant upon the experiment.

During the exposure in the heat chamber the animals were either confined in a cage or tied, so that while they had a certain amount of freedom they could not come in contact with the stove or otherwise injure themselves or the apparatus. At the lower temperatures they usually rested quietly or slept unless disturbed for purposes of observation. At 45° and 50°C. they were somewhat restive during the first few minutes of the exposure but later became quiet.

All of the blood samples were drawn from the jugular vein by venepuncture, in no case was venesection practiced.

For the convenience of the reader the results are presented in the form of graphs. These have been plotted from the means calculated from the various values obtained at the end of each interval and at each temperature. In order to obtain some idea of the mean average condition of the animals at the beginning of the experiments, the means and the mean standard deviations for all of the initial values of the several determinations were calculated. These mean initial

values we have used as a base with which to compare the effects of the experimental procedures. This was done by adding algebraically to each of the means of an experimental series an amount sufficient to make the initial value of that series equal to the mean of all the corresponding initial determinations. Exactly what was done will be made clear by reference to table 1. In this table are shown the means obtained for the initial determinations and those for a single

TABLE 1

*To illustrate the method used in tabulation and the derivation of the data used in plotting the curves*

| | BODY WEIGHT | RECTAL TEMPERATURE | PER 100 CC. OF BLOOD | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | $CO_2$ content | $CO_2$ capacity | $O_2$ content | $O_2$ capacity | Glucose | Total solids |
| **Initial values, 47 observations** | | | | | | | | |
| | *Kg* | *°C.* | *cc.* | *cc.* | *cc.* | *cc.* | *mgm.* | *mgm.* |
| Mean values | | 38.7 | 46.7 | 62.5 | 17.7 | 25.1 | 91 | 21.7 |
| Mean standard deviation | | | ±4.8 | ±4.9 | ±3.4 | ±3.1 | ±11 | ±2.1 |
| **Chamber temperature 20°C. Relative humidity 0.46; 12 observations** | | | | | | | | |
| Initial mean | 14.8 | 38.9 | 45.2 | 67.0 | 18.4 | 25.3 | 92 | 22.2 |
| Correction added | | −0.2 | 1.5 | −4.5 | −0.7 | −0.2 | −1 | −0.5 |
| Modified mean | | 38.7 | 46.7 | 62.5 | 17.7 | 25.1 | 91 | 21.7 |
| Mean standard deviation | | | ±5.4 | ±3.3 | ±3.0 | ±3.5 | ±12 | ±2.0 |
| 2nd hour, mean | 14.8 | 39.0 | 45.8 | 66.9 | 19.0 | 25.7 | 87 | 22.4 |
| Modified mean | | 38.6 | 47.3 | 62.4 | 18.3 | 25.5 | 86 | 21.9 |
| Mean standard deviation | | | ±5.6 | ±3.3 | ±3.0 | ±3.9 | ±17 | ±1.8 |
| 4th hour, mean | 14.7 | 38.2 | 46.0 | 67.7 | 18.8 | 25.6 | 85 | 22.8 |
| Modified mean | | 38.0 | 47.5 | 63.1 | 18.1 | 25.4 | 84 | 22.3 |
| Mean standard deviation | | | ±5.3 | ±3.7 | ±3.1 | ±3.0 | ±16 | ±2.5 |
| 6th hour, mean | 14.7 | 38.3 | 46.0 | 67.9 | 18.8 | 25.5 | 83 | 22.3 |
| Modified mean | | 38.1 | 47.5 | 63.4 | 18.1 | 25.3 | 82 | 21.8 |
| Mean standard deviation | | | ±5.2 | ±3.6 | ±3.4 | ±3.4 | ±15 | ±1.9 |

temperature—20°. Together with the means are shown the correction which was added and the modified mean so obtained. This last was used in all cases in plotting the curves shown. (cf. Scott and Ford (9).)

The formula used in calculating the mean standard deviation was:

$$\sigma = \frac{\sqrt{\frac{\Sigma (d)^2}{N-1}}}{\sqrt{N}}$$

where $\sigma$ = the mean standard deviation; $d$ = the arithmetical difference between the individual determinations and the mean for the series, and $N$ the number of observation.

It is planned to publish shortly the complete data obtained in this research together with additional material as a bulletin of the United States Public Health Service. It is hoped that those interested in the detailed results will make use of this publication.

TABLE 2

*To show the number of observations made and the individual dogs used in the first series, embracing determinations of oxygen content and capacity, carbon dioxide content and capacity, blood sugar and total solids*

| CONDITIONS | | OBSERVATIONS ON DOG | | | | | | TOTAL OBSERVATIONS |
|---|---|---|---|---|---|---|---|---|
| Temperature | Mean relative humidity | E | H | J | M | P | Q | |
| °C. | | | | | | | | |
| Initial | | 7 | 4 | 5 | 10 | 10 | 10 | 46 |
| 20 | 46 | 2 | 2 | 2 | 2 | 2 | 2 | 12 |
| 30 | 43 | 2 | 2 | 2 | 2 | 2 | 2 | 12 |
| 40 | 39 | 2 | 0 | 1 | 2 | 2 | 2 | 9 |
| 45 | 35 | 1 | 0 | 0 | 2 | 2 | 2 | 7 |
| 50 | 28 | 0 | 0 | 0 | 2 | 2 | 2 | 6 |

TABLE 3

*To show the number of observations made and the individual dogs used in the second series, embracing determinations of the concentration of hydrogen ion and carbon dioxide content of the oxalated plasma*

| TEMPERATURE | OBSERVATIONS ON DOG | | | | TOTAL OBSERVATIONS |
|---|---|---|---|---|---|
| | M | P | S | T | |
| °C. | | | | | |
| 20 | 1 | 1 | 1 | 1 | 4 |
| 30 | 1 | 1 | 1 | 1 | 4 |
| 40 | 1 | 1 | 1 | 1 | 4 |
| 45 | 2 | 2 | 2 | 2 | 8 |
| 50 | 2 | 2 | 2 | 2 | 8 |

EXPERIMENTAL RESULTS AND DISCUSSION. The total number of experiments which were carried out together with the experimental conditions to which the animals were subjected as well as the individual dogs which served as subjects are indicated in tables 2 and 3.

I. *The effects of various environmental temperatures upon the body temperature.* The changes in the body temperature during exposure to the several conditions studied are shown graphically in figure 1.

The mean body temperature for forty-seven initial observations was 38.7°C. It was found that the body temperature fell slightly for a time and then remained at a fairly constant level for the remainder of the period of observation. This agrees with the results reported by the New York State Commission of Ventilation for men when they were subjected to similar conditions (10).

At environmental temperatures of 30° the history for the dogs was much the same as for 20° except that the fall in body temperature was not quite so marked. The New York State Commission

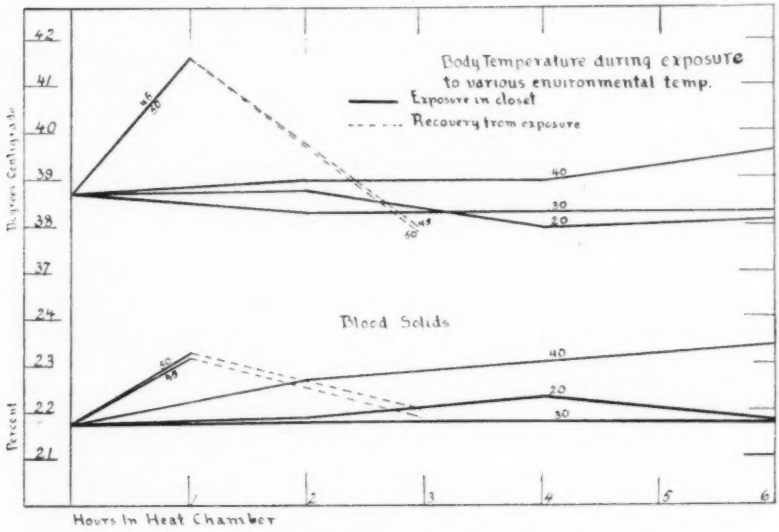

Body Temperature during exposure to various environmental temp.
Exposure in closet
Recovery from exposure
Blood Solids
Hours In Heat Chamber

Fig. 1. Curves to show the relation between various environmental temperatures and the temperature of the body and the total solids of the blood of dogs.

of Ventilation reported a slight rise of body temperature in men when they were exposed to an environmental temperature of 30°, though in their case the humidity was 80 per cent, much higher than in any of our experiments. The explanation of this behavior is not clear to us although it may possibly be related to the normal diurnal variations in the body temperature as our observations and those of the Commission were begun in the morning and continued for some time through the day. Or again it may be related to the decreased muscular activity which accompanies confinement in the chamber and to the fact that no food was taken during the course of the observations. This latter interpretation seems the more probable

in view of the fact that the diurnal curves usually shown are highest in the late afternoon and hence vary in the sense opposite to that observed by us.

A consideration of the work of Rubner (11) is of interest at this point, for he has shown that at 20°C. the dog manifests no increase in the rate of metabolism, and that this condition of unchanging metabolism persists while the environmental temperature is raised to at least 30°. He has further demonstrated that in the fasting animal the minimum energy release occurs at temperatures of from 30° to 35°C. This might be called the basal energy requirement, or the minimum energy release compatible with mammalian life.

When the temperature of the chamber is raised to 40°, the response is quite different. Here we find a *rise* of 1 degree in body temperature during the six-hour period of observation, and as a rise was apparent at the end of the first hour of exposure there was no evidence of an initial fall as observed in the two previous cases. The temperature remains constant during the middle period, the rise being confined to the first and last two-hour periods. Whether or not this is of significance we are not prepared to say.

At temperatures of 45° and 50° a very marked rise of the rectal temperature was noted, which apparently began at once. In fact this rise was so sharp that at the end of one hour the body temperature had risen to such a point that it was not deemed safe to continue the experiments at these temperatures for a longer time. On removal of the animal from the chamber the body temperature rapidly fell and two hours after removal the body temperature was subnormal, or about 36°. This subnormal temperature reminds one of the conditions in certain stages of heat stroke in man.

It will be noted that the body temperature is the same whether the animal had been exposed to an environmental temperature of 45° or 50°. This remarkable fact of a similar physiological response to conditions of different severity was noted in some of the other factors studied it being especially marked for those having to do with the carbon dioxide. The organism seems to yield with increasing rapidity as the strain impressed upon it becomes more severe, until a certain limit is reached at which point great resistance is interposed by the organism against any further change. If the strain is increased so that the organism is pushed beyond this point, it will recover with great difficulty, if at all, unless external aid is given. This same type of physiological limitation was noticed by Henderson and Haggard (12) during their work on low levels of carbon dioxide and alkali reserve induced by ether. It was also noticed by Britton in his studies on cooling. In order to lower the temperature of his

animals below what might be called the critical point he found it necessary to subject them to anesthesia until it had been passed, after which the anesthesia was no longer necessary and the body temperature continued to fall so long as the animal was exposed to an environmental temperature lower than the body temperature.

II. *The effects of various environmental temperatures upon the oxygen content and upon the amount of hemoglobin in the blood.* Considering first the oxygen capacity (hemoglobin) we find very little variation throughout the series. In fact, all of the mean values are within the limits of the mean standard deviations of the 20° series and so from a purely statistical standpoint are without significance. The direction of the slight variations which do occur fits so nicely with other circumstances, however, that one hesitates to dismiss them without further discussion. (Fig. 2.)

The changes noted at 20° and 30°, if not entirely fortuitous, may be an example of the diurnal changes studied for man and the goat by Dreyer, Bazett and Pierce (14). At temperatures of 40° and above there is a slight tendency for the oxygen capacity to increase, this tendency becoming somewhat more marked as the environmental temperature rises. This increase is paralleled by an increase in the total solids, and we have related it simply to an increase in the concentration of the blood due to the excessive evaporation of water, accompanied by an inability of the water reservoirs to supply water at a rate sufficient to meet the demand made upon them at these high temperatures.

The fact that there was no increase in the oxygen capacity during the first two-hour period of exposure to a temperature of 40° would seem to bear out this interpretation. It might fairly be assumed that at this temperature the loss would not be so rapid but that sufficient time would be permitted for equilibration and that there had not yet occurred so great a loss that the available store of water had become seriously depleted. The later rise, then, was probably due to an actual depletion of the available water below a limit where the original concentration of the blood could be maintained.

At 45° and 50° it may be presumed that while the actual quantity of water which has been lost from the tissues during the first hour of exposure is not of itself serious, the rate of loss of water from the blood is so rapid that the organism is unable to maintain the original concentration of the blood. This interpretation is further supported by the rapid return of the oxygen capacity and of the total solids to their original level during the first two hours after the animal was removed from the chamber.

A very slight fall is noted in the oxygen capacity of the animals exposed to 30°. A comparison of this fall with the mean standard deviation of the 20° series, however, indicates that it is too small to be of significance from the standpoint of statistics. Some biological considerations, on the contrary, seem to show that it may have some importance; but this evidence is not sufficient to warrant discussion at the present time.

There is a fairly marked fall in the oxygen content of the venous blood of animals exposed to a temperature of 30° as compared with

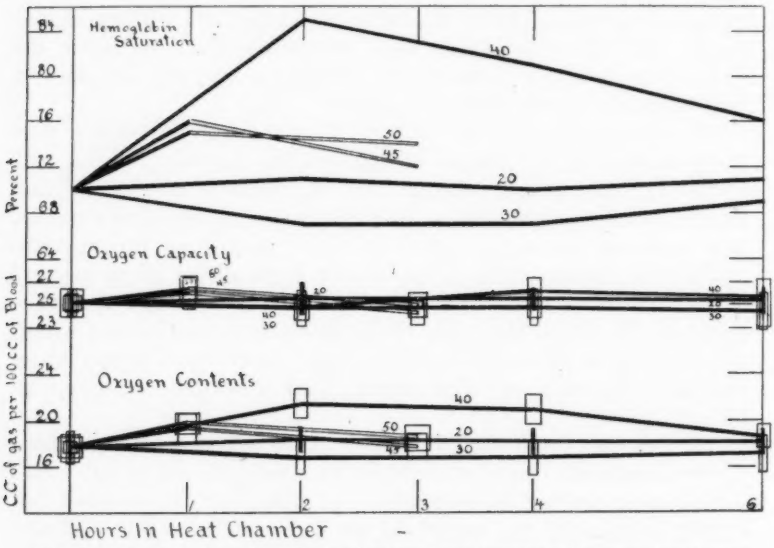



Fig. 2. Curves to show the effects of various environmental temperatures upon the oxygen capacity, oxygen content and the percentage of oxygen saturation of the venous blood of dogs.

that of animals exposed to a temperature of 20°. This drop we believe to be a reflection of the manner in which the animals respond to the two temperatures. At 30° and the humidities at which we were working, there seems to be nearly an equilibrium between the heat generated in the basal metabolism and the heat lost to the environment. (Cf. Voit (15).) The animals are quick to take advantage of this and stretch themselves out and take life easily. Hyperpnea has not yet become necessary to keep the temperature of the body down. All muscular movements, and consequently heat generation, are at a minimum. The net result is a considerably

reduced aeration of the blood, as well as a reduced circulation and consequently a reduced oxygen content, especially of the venous blood.

At 40° there is a considerable increase in the rate of respiration, and correlated with this, a rather marked increase in the oxygen content of the blood. The rate of increase in the oxygen content is slightly greater at 45° and 50°, respectively, than it is at 40°, but the increased rates are not nearly so great as might be expected when one compares them with that occurring between 30° and 40°. There are at least two factors concerned in this: First, while the rate of respiration continues to increase as the environmental temperature continues to rise it becomes progressively shallower (fig. 3), so that while the efficiency of the respiratory apparatus as a cooling mechanism may rise because of the increased passage of air over the membranes of the mouth and throat and a consequent increase in the vaporization of water, its efficiency as a means of aerating the blood does not increase in anything like the same ratio, if at all. Second, with the increased body temperature which accompanies exposure to these high environmental temperatures the rate of metabolism is increased and this would of course be reflected in a decreased oxygen content of the venous blood provided that there was not at the same time a considerable increase in the amount of oxygen carried in the arterial blood, which from the evidence just discussed, is probably not the case. The curves showing the percentage of saturation (fig. 2) are very satisfactorily explained on this hypothesis.

III. *The effects of various environmental temperatures upon the carbon dioxide content and upon the alkali reserve of the blood.* There is no change in the carbon dioxide capacity of the blood during an exposure of six hours to a temperature of 20° or of 30°, as is shown in figure 4, where it will be seen that the two curves are identical within the limits of the mean standard deviation and that both are horizontal.

When the environmental temperature is raised to 40° there is a fairly rapid fall during the first four hours and a somewhat slower fall during the remaining two hours. At 45° and 50° there is a very rapid depletion of the alkali reserve as shown by the carbon dioxide capacity, this depletion being almost identical in degree for the two temperatures. This is a very good illustration of the critical point previously mentioned in the discussion of body temperature. We believe that the changes noted result directly from the equilibration necessitated by the washing out of the carbon dioxide which, in turn, is caused by the hyperpnea due to the high temperatures—a hyperpnea which may even become dyspnea if the exposure is long continued.

The carbon dioxide content (fig. 5) gives a similar picture, except that here a slight rise is noted in the animals exposed to an environmental temperature of 30°. This probably results from the same cause as the corresponding slight depression of the oxygen content

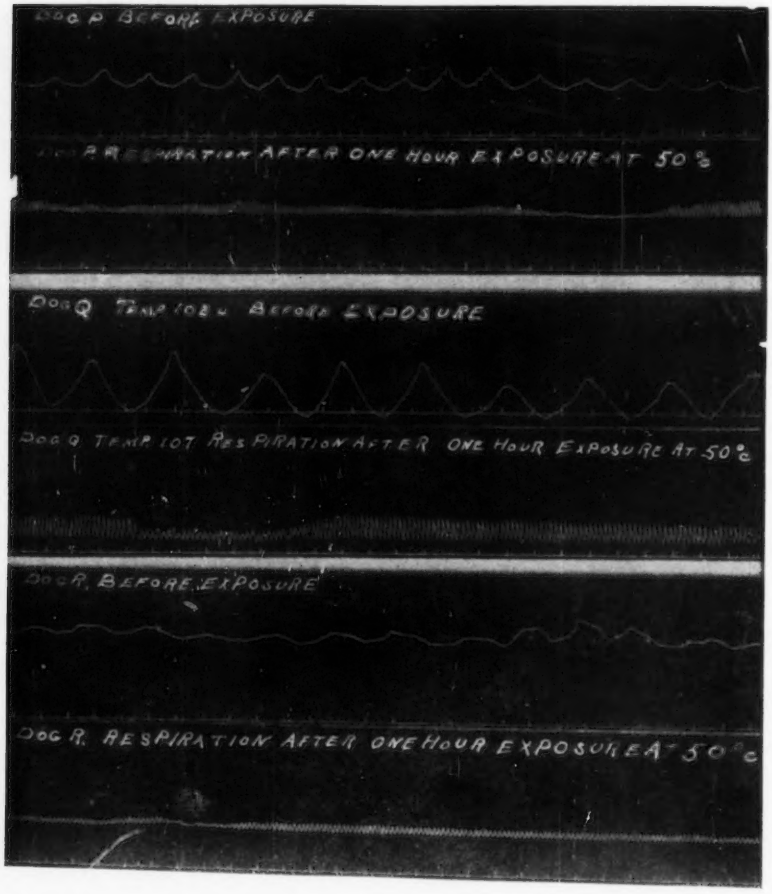

DOG P BEFORE EXPOSURE
RESPIRATION AFTER ONE HOUR EXPOSURE AT 50°
DOG Q TEMP 101.4 BEFORE EXPOSURE
DOG Q TEMP 107 RESPIRATION AFTER ONE HOUR EXPOSURE AT 50°C
DOG R. BEFORE EXPOSURE
DOG R. RESPIRATION AFTER ONE HOUR EXPOSURE AT 50°C

Fig. 3. Pneumographic tracings to show the relative rate and depth of respiration of dogs subjected to environmental temperatures of 20° and 50°C.

which was noted for the same condition, i.e., a small decrease in the rate of metabolism, which would be, under these conditions, at its lowest ebb as shown by Rubner and others. This decreased metab-

olism would result in a slight lowering of the rates of respiration and circulation.

IV. *The effects of various environmental temperatures upon the hydrogen-ion content of the plasma.* The hydrogen-ion concentration

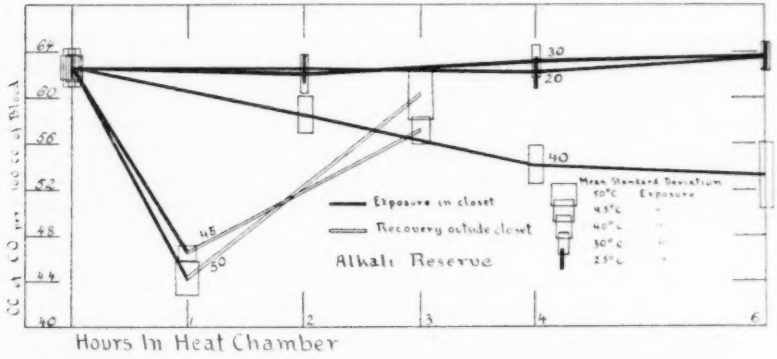



Fig. 4. Curves to show the effects of various environmental temperatures upon the alkali reserve of the blood of dogs as determined by the carbon dioxide capacity.

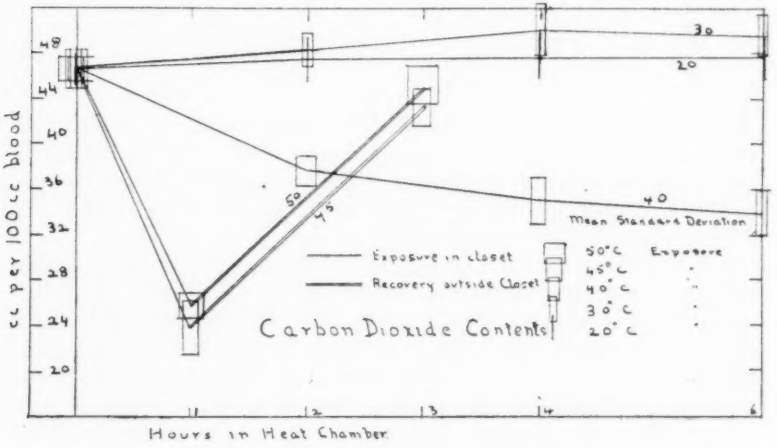



Fig. 5. Curves to show the effects of various environmental temperatures upon the carbon dioxide content of the venous blood of dogs.

expressed as pH remains constant for at least six hours when the animals are exposed to temperatures of 20° or 30° (table 4) and falls within the normal acid-base balance, or area 5 of the Van Slyke

chart (16). At 40° with an increased rate of respiration and the resultant fall in the carbon dioxide content and the alkali reserve, we find that the blood index has passed from area 5 to area 6 of the Van Slyke chart, or into the region of compensated carbon dioxide deficit. The alkali reserve likewise falls thus preventing an abnormal alkalinity. As the strain becomes greater with environmental temperatures of 45° and 50°, the pH increases from the normal of 7.55 to 7.79 and to 7.84 for the two temperatures respectively. The carbon dioxide of the plasma has dropped to 29.9 and to 26.3 volumes per cent and the plasma index has passed into area 2 or 3 of the Van Slyke chart or into the region of uncompensated carbon dioxide deficit as the result of an excessive loss of carbon dioxide. This loss of carbon

TABLE 4

*To show the effect of various environmental temperatures upon the concentration of the hydrogen ion and carbon dioxide content of the plasma*

| TEMPERATURE OF CHAMBER | BEFORE EXPOSURES | | AFTER ONE HOUR EXPOSURE | | AFTER SIX HOURS EXPOSURE | |
|---|---|---|---|---|---|---|
| | pH | $CO_2$ content | pH | $CO_2$ content | pH | $CO_2$ content |
| °C. | | | | | | |
| 20 | 7.57 | 52.4 | 7.57 | 52.4 | 7.57 | 55.4 |
| 40 | 7.57 | 52.4 | 7.56 | 46.6 | 7.56 | 39.5 |
| 45 | 7.57 | 52.4 | 7.79 | 29.89 | | |
| 50 | 7.57 | 52.4 | 7.83 | 26.3 | | |

dioxide was induced by an increase of the respiratory rate which was evidently brought about by some stimulus other than an increased concentration of the hydrogen-ion. The same condition, also accompanied by hyperpnea, has been observed by Bazett and Haldane (17) in man when immersed in warm baths.

Kahn (18) and Barbour (19) are of the opinion that the mechanism causing the increased rate of respiration is the increased temperature of the blood, that is, it is the direct result of the increased body temperature. It is known that cellular activities in general increase, within limits, with the temperature and there is no apparent reason for excepting the respiratory center from this category. It was at first thought that the stimulus might be due to a local accumulation of hydrogen-ion within the cells of the respiratory center itself, which might occur as a part of a general tissue anoxemia depending upon the increased stability of the oxyhemoglobin at low carbon dioxide concentrations. (Cf. Bohr (20).) If such an anoxemia should in truth exist one would expect to find it indicated by an accumulation of lactic acid in the blood, but a careful search has failed to show the slightest increase

of this acid in the blood of animals exposed to high temperatures, over the amount occurring under ordinary conditions. It is difficult for us to imagine a significant acidosis occurring in the tissues without its being mirrored in the blood stream.

Our work does not permit us to accept the suggestion of Hill and Flack (21) and of Mayer (22) that fatal termination from over-heating is due to an accumulation of acid. We found a decreased rather than an increased hydrogen-ion concentration in the plasma of all of our animals which recovered from exposure to severe conditions and in none did we find any indication of increased lactic acid. From two animals, Q and R, which died shortly after removal from the chamber after having been exposed to rather severe conditions, blood was drawn only a few minutes before death. The mean values obtained were:

| | | | |
|---|---|---|---|
| Oxygen content | 5.9 | Carbon dioxide content | 30.5 |
| Oxygen capacity | 23.6 | Carbon dioxide capacity | 36.7 |

Hydrogen-ion determinations were not made, but from the Henderson nomogram (23) the pH may be presumed to have been about 7.4, which would not indicate acidosis. (Compare Henderson (24).)

Since the respiratory efforts gave every indication of the passage of at least a normal volume of air up to the time that the samples were taken we have interpreted the changes which occurred as indicating circulatory rather than respiratory failure, this having been induced by the overload thrown on the circulatory mechanism in the effort to keep the body temperature down.

V. *The relation between the concentration of the sugar in the blood and the temperature of the environment.* The changes in the concentration of the sugar in the blood (fig. 6) are rather hard to explain in a manner which is consistent for all of the conditions studied. At 20° there is a fairly uniform fall throughout the six-hour period of observation. This is in entire agreement with the observation of Scott and Hastings (25) and can hardly be related to the ingestion of food since sufficient time was allowed for this factor to become constant before the first sample was drawn. The only explanation which occurs to us is that the animals were becoming progressively quieter as the experiments proceeded and consequently were mobilizing less and less sugar. Whether such a decrease in the rate of mobilization of sugar is the direct result of lessened excitement, or whether it is due to the operation of an unknown factor which tends to equilibrate the concentration of sugar in the blood with the metabolic requirements of the organism for sugar, is a matter which we are unable to discuss at present. Aside from this factor which may be assumed, for the time being, to have been constant

throughout the research, it is to be noted that when the corresponding periods of the different series are compared a rough agreement between the concentration of the sugar in the blood and the body temperature is noted. The 45° series forms an exception to the rule, however, for at this environmental temperature the body temperature is almost identical with that which occurs when the animals are exposed to a temperature of 50°; while the concentration of sugar occupies a position midway between that found at 40° and that at 50°. This rise in the blood sugar is then not exclusively dependent upon the body temperature but seems also to be associated with the environmental temperature

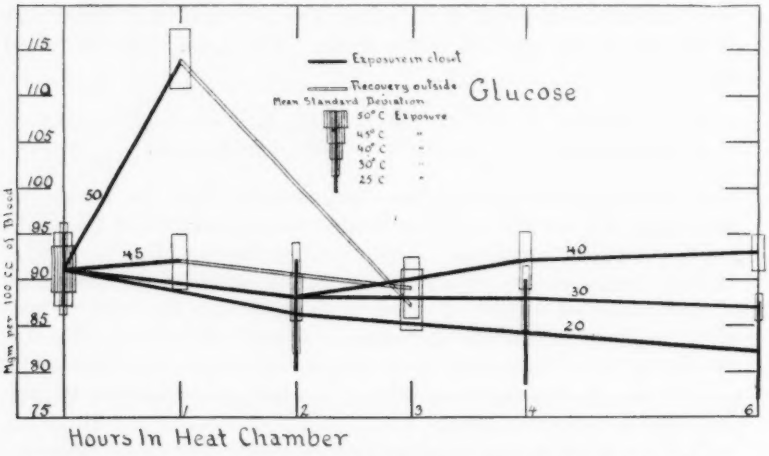



Fig. 6. Curves to show the effects of various environmental temperatures upon the concentration of glucose in the venous blood of dogs.

in a more direct manner. It is apparently not dependent upon any emotional disturbances resulting from the exposure to the higher temperatures. We hope to be able to give further evidence regarding this phase in a later paper. Lépine (26) attributes the hyperglycemia which is occasionally present in fever to an irritation of the fourth ventricle by the fever toxins. The increase in the concentration of sugar which Freund and Marchand (27) report in fever is only of such a degree that it may be accounted for by changes in the concentration of the blood. We are not, however, able to explain our results in this manner since we found the increase in the sugar to exceed that of the total solids; in one case there was an increase in the sugar of 100 per cent while at the same time the total solids increased only 25 per cent.

VI. *The relation between the total solids of the blood and the environmental temperature.* The total solids of the blood tend to increase as the environmental temperature rises and we have been unable to observe the dilution mentioned by Barbour (28) in the report of his experiments with hot and cold baths. Our results seem to be more in line with the results which he obtained in coli fever (29). There is no doubt some mechanism by which the total blood volume is regulated, and our results should be considered as indicating some interference with the activity of this mechanism. At high temperatures the rate of replacement of water cannot keep pace with the rate of its loss and a certain amount of concentration results. There is, however, a fairly large factor of safety for it is not until the concentration of the blood approaches 25 per cent that pathological symptoms occur from this cause.

## SUMMARY

1. During an exposure of six hours to an environmental temperature of 20° or of 30° there was a drop in body temperature, probably due to a decrease of muscular activity. At 40° there was an increase of 1 degree in body temperature without an initial drop At 45° and 50° the body temperature rose within an hour to such a height that it was deemed unsafe to continue the experiments at these temperatures for a longer time.

2. The oxygen capacity of the blood showed no change during an exposure to the different temperatures that cannot be accounted for by the diurnal changes in the hemoglobin or by the concentration of the blood due to the excessive evaporation of water.

3. The oxygen content of the blood remains unchanged at 20°, but shows a drop at 30° which is probably associated with the low rate of metabolism at this temperature. At 45° and 50° there is a slight increase in the oxygen content due to the increased aeration of the blood at these temperatures, but this increase is not in direct proportion to the increased passage of air over the membranes of the mouth and throat.

4. At temperatures of 20° and 30° the carbon dioxide capacity of the blood remains unchanged, while at 40° there is a sharp fall during the first four hours followed by a slower fall during the next two hours. At temperatures of 45° and 50° there is a rapid depletion of the carbon dioxide capacity from the beginning, which is almost identical for these two temperatures.

5. The carbon dioxide content follows the capacity except that at 30° there is a slight rise for the same reason that the oxygen content falls.

6. The hydrogen-ion content of the plasma remains unchanged during an exposure of the animal to temperatures of 20°, 30° and 40° for six hours, but decreases at temperatures of 45° and 50° due to the excessive pulmonary ventilation at these temperatures with the consequent washing out of the carbon dioxide and without a compensatory loss of alkali from the blood.

7. The concentration of blood sugar falls during an exposure to temperatures of 20° and 30°. This fall is probably associated with inactivity of the animal during the course of the experiment. At 40° it falls during the first two hours, to increase during the following four hours. At 45° no change was noted during an hour's exposure while at 50° there was a sharp rise during this time.

8. The blood solids at 20° and 30° showed only the usual diurnal changes, while at 40°, 45° and 50° the concentration of the blood increased with the environmental temperature, no initial drop having been observed.

## BIBLIOGRAPHY

(1) Van Slyke: Journ. Biol. Chem., 1917, xxx, 347.
(2) Van Slyke and Stadie: Journ. Biol. Chem., 1921, xlix, 1.
(3) MacLean: Biochem. Journ., 1919, xiii, 135.
(4) Hastings and Hopping: Proc. Soc. Exper. Biol. and Med., 1923, xx, 254.
(5) Brown: Journ. Amer. Chem. Soc., 1922, xliv, 423.
(6) Scott and Flinn: Journ. Biol. Chem., 1923, l, Proc. Soc., 32.
(7) Cullen: Journ. Biol. Chem., 1922, lii, 501.
(8) Hastings: Jour. Ind. Eng. Chem., 1921, xiii, 1056.
(9) Scott and Ford: This Journal, 1923, lxiii, 520.
(10) Ventilation Report of the New York State commission of Ventilation; 1923, p. 51 et seq.
(11) Rubner: Energiegesetz. 1902, pp. 105–137.
(12) Henderson and Haggard: Journ. Biol. Chem., 1918, xxxiii, 333.
(13) Britton: Quart. Journ. Exper. Physiol., 1922, xiii, 55.
(14) Dreyer, Bazett and Pierce: Lancet, 1920, ii, 588.
(15) Voit: Zeitschr. f. Biol., 1901, xli, 125.
(16) Van Slyke: Journ. Biol. Chem., 1921, xlviii, 153.
(17) Bazett and Haldane: Journ. Physiol., 1921, lv, 125.
(18) Kahn: Arch. f. Physiol., Supple. 1904, 31.
(19) Barbour: Physiol. Rev., 1921, i, 295.
(20) Bohr, Hasselbach and Krogh: Skand. Arch. Physiol., 1907, xvi, 390.
(21) Hill and Flack: Journ. Physiol., 1909, xxxviii, Proc. lvii and lxi.
(22) Mayer: Carnegie Pub. no. 252.
(23) Henderson: Journ. Biol. Chem., 1921, xlvi, 411.
(24) Henderson: This Journal 1910, xxv, 397.
(25) Scott and Hastings: Proc. Soc. Exper. Biol. and Med., 1920, xvii, 120.
(26) Lepine: Rev. Med., 1915, xxxiv, 657.
(27) Freund and Marchand: Arch. f. Exper. Path. u. Physiol., 1913, lxxiii, 276.
(28) Barbour: Proc. Soc. Exper. Biol. and Med., 1921, xviii, 184.
(29) Barbour and Howard: Proc. Soc. Exper. Biol. and Med., 1920, xvii, 148.

# ON ABSORPTION FROM THE CLOACA IN BIRDS

N. C. SHARPE

*From the Department of Pharmacology, University of Toronto*

Received for publication June 18, 1923

Some years ago (1), 1911, while working in this department on the urinary secretion of birds, it was noticed that while birds kept in metabolism cages excreted practically no fluid urine, but only small amounts of thick curdy fluid were ejected from the anus, yet the urine from ureters was clear and limpid. These facts suggested reabsorption of water by the cloaca. One or two preliminary experiments confirmed this, and it was reported. A more careful examination of this absorption together with a study of the possible absorption of salts seemed to be indicated, and has been carried out in the experiments reported in this paper.

Hens were used in all the experiments. Male birds were found more suitable for operation than female though both were used. They were in all cases anesthetized with urethane, given by subcutaneous injection, 1.5 gram per kilo, followed by ether during the time of operation. Artificial respiration was needed in very few cases. The cloaca was exposed and tied as close to the entrance of the two blind coecal pouches as possible. This gave a tube for experimental work varying in length from 5 to 8 cm. A glass cannula was inserted into the anal aperture. Metal cannulae were inserted in some cases into the ureters; in other cases no urine was collected in this way and the ureters and genital tubes were tied and cut to prevent their contents entering the cloaca. Blood for analysis was obtained from a cannula in the external jugular vein. Changes in surface temperature were diminished by using an electric lamp and covering with cloths. The cloaca was washed out thoroughly with the fluid to be tested and then a known amount was left in the cloaca. This was withdrawn for a given period and analyzed. In some cases fresh fluid was put in for a further period. The upper portion of the cloaca used in these experiments has a fairly smooth wall, whereas nearer the anus there occur three heavy main folds and closer still to the anus the folds are numerous and irregular. When empty this lower portion is finely wrinkled in appearance and suggests an absorbing service somewhat similar to that found in the small intestine. Certain experiments

suggested that if the fluid were put into the gut under pressure even of 4 or 5 cm. of water, absorption did not take place as readily as with a pressure of say 1 cm.

The various solutions used for absorption were colored in all cases with hemoglobin. This was prepared by mixing hens' blood corpuscles with water, and centrifuging to remove any unlaked cells or stroma. In two cases a solution of phenolsulphonephthalein was used instead of hemoglobin. The percentage of sodium chloride varied from 0.02 to 0.86 per cent.

The use of hemoglobin was suggested in the paper by Shoji (2) on absorption from the urinary bladder. By comparing the concentration of hemoglobin in the solution removed from the cloaca with that in the original solution an estimation of the amount of water absorbed or excreted into the cloaca could be made and this served as a check upon the amounts that could be obtained by draining the cloaca. The agreement in the two cases was very good. As it was possible that the cloacal tissue might change the hemoglobin solution, a check was made upon dead animals or on animals with poor circulation and in which no absorption appeared to take place. In such cases the hemoglobin remained unaltered and no chloride or water was absorbed.

The following are typical protocols selected from some twenty successful experiments.

*No. 5.* November 16, 1922. Female, 1 kgm.

Anesthetic: Urethane subcutaneously. Ether during operation.

Fluid used: Hemoglobin solution + NaCl. Concentration 0.62 per cent NaCl.

Cannulae in ureters, cloaca, anus and jugular vein.

0 hr. Blood chlorides 542 mgm. NaCl per 100 gram. Urine flowing from ureter cannulae.

0 hr. 30 min. Urine 7 cc. contains chlorides 89 mgm. NaCl per 100 cc.

2 hr. 30 min. Blood chlorides 565 mgm. NaCl per 100 gram 13 cc. fluid into cloaca. Chlorides 81 mgm. in 13 cc.

4 hr. 30 min. Withdrew fluid from cloaca—11.3 cc. Chlorides 67 mgm. in 11.3. Blood chlorides 594 mgm. per 100 cc. 15.3 cc. fluid into cloaca. Chlorides: 95 mgm. in 15.3.

5 hr. 30 min. Withdrew fluid from cloaca—15.3 cc. Chlorides 94 mgm. in 15.3.

| | WATER | CHLORIDES |
|---|---|---|
| | *cc.* | *mgm.* |
| 2 hours | 1.7 | 14 |
| 1 hour (bird in poor condition) | 0 | 1 |
| 3 hours (totals) | 1.7 | 15 |

ABSORPTION FROM CLOACA

*No. 24.* March 27, 1923. Male, 2 kgm.

Anesthetic: Urethane subcutaneously and ether during operation.

Fluid used: Clear NaCl solution.

Analysis: 0.835 per cent NaCl, 0.347 per cent Na.

Cannulae in ureters, cloaca, anus and jugular vein.

0 hr. 5 cc. fluid left in cloaca after washing out. Ureter cannulae filling. In 5 cc. fluid there are 42 mgm. NaCl and 17 mgm. K. Blood: Chlorides 479 mgm. NaCl per 100 grams.

1 hr. Urine 30.5 cc. Withdrew fluid from cloaca 3.4 cc.

Chlorides: 25 mgm. in 3.4 cc. Sodium 11 mgm. in 3.4 cc. 5 cc. fluid into cloaca.

1 hr. 30 min. Urine 9.5 cc.

3 hr. Withdrew fluid from cloaca 2.7 cc.

Analysis: Chlorides 22 mgm. in 2.7 cc. Sodium 7.5 mgm. in 2.7 cc.

5 cc. fluid into cloaca.

4 hr. Withdrew fluid from cloaca 4.1 cc.

Analysis: Chlorides 29 mgm. in 4.1 cc.
Sodium 13 mgm. in 4.1 cc.

Blood: Chlorides 565 mgm. NaCl per 100 grams.

*Absorption from cloaca*

| | WATER | CHLORIDES | SODIUM |
|---|---|---|---|
| | *cc.* | *mgm.* | *mgm.* |
| 1st hour | 1.0 | 17 | 6.0 |
| Next 2 hours | 2.3 | 20 | 9.5 |
| 1 hour | 0.9 | 13 | 4.0 |
| 4 hours (totals) | 4.8 | 50 | 19.5 |

*No. 29.* May 3, 1923. Male, 2 kgm.

Anesthetic: Subcutaneous urethane and ether during operation.

Fluid used: Hemoglobin solution $+KCl+CaCl_2$ Concentration: 0.17 per cent NaCl, 0.03 per cent K, 0.007 per cent Ca.

Cannulae in cloaca, anus and jugular vein. Ureters tied.

0 hr. 5 cc. fluid into cloaca after washing.

Analysis: Chlorides 8.5 mgm. NaCl.
Potassium 1.5 mgm. K.
Calcium 0.38 mgm. Ca.

0 hr. 15 min. Blood analysis: Chlorides 465 mgm. NaCl per 100 grams whole blood.
Sodium 385 mgm. Na per 100 gram whole blood.
Potassium 11 mgm. K per 100 gram whole blood.
Calcium 13 mgm. Ca per 100 gram whole blood.

2 hr. Withdrew fluid from cloaca— 2 cc.

Analysis: Chlorides 8.5 mgm. NaCl.
Potassium 0.28 mgm. K.
Calcium 0.17 mgm. Ca.

Blood analysis: Chlorides 494 mgm. NaCl per 100 gram whole blood.
Sodium 327 mgm. Na per 100 grams whole blood.
Potassium 15 mgm. K per 100 grams whole blood.
Calcium 10 mgm. Ca per 100 grams whole blood.

2 hr. 5 cc. fluid into cloaca.
4 hr. Withdrew fluid from cloaca. 1.9 cc.
Analysis: Chlorides 7.5 mgm. NaCl.
Potassium 0.35 mgm. K.
Calcium 0.14 mgm. Ca.
5 cc. fluid into cloaca.
5 hr. Withdrew fluid from cloaca. 3.0 cc.
Analysis: Chlorides 8.5 mgm. NaCl.
Potassium 1.3 mgm. K.
Calcium 0.3 mgm. Ca.
Blood analysis (on serum owing to increased viscosity).
Chlorides 629 mgm. NaCl per 100 cc. Serum.
Sodium 388 mgm. Na per 100 cc. serum.
Potassium 31 mgm. K per 100 cc. serum.
Calcium 18 mgm. Ca per 100 cc. serum.

*Absorption from cloaca*

| | WATER | CHLORIDES | SODIUM | POTASSIUM | CALCIUM |
|---|---|---|---|---|---|
| | *cc.* | *mgm.* | | *mgm.* | *mgm.* |
| 1st 2 hours | 3.0 | 0 | | 1.2 | 0.2 |
| Next 2 hours | 3.1 | 1 | | 1.1 | 0.2 |
| Next 1 hour | 2.0 | 0 | | 0 | 0 |
| Totals, 5 hours | 8.1 | 1 | | 2.3 | 0.4 |

In the following table the absorption from the cloaca has been calculated on a five-hour basis.

| EXPERIMENT NUMBER | CONCENTRATION OF TEST SOLUTION | CHANGES IN CONTENT OF CLOACA | | | | |
|---|---|---|---|---|---|---|
| | | Water | Chlorides NaCl | Na | K | Ca |
| | | *cc.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| M 16 | 0 NaCl | −11.0 | +8 | | | |
| M 13 | 0.026% NaCl | −5.6 | +26 | | | |
| M 19 | 0.04% NaCl | −2.1 | +1.4 | | | |
| M 14 | 0.07 NaCl | −9.1 | −7.4 | | | |
| F 5 | 0.62% NaCl | −2.8 | −25 | | | |
| F 7 | 0.66% NaCl | −1.1 | −6 | | | |
| F 8 | 0.66% | 0 | 0 | | | |
| M 20 | 0.81 | −4.6 | −37 | | | |
| M 21 | 0.86 | −2.4 | −19 | −17 | | |
| M 22 | 0.81 | −3.2 | −30 | −32 | | |
| M 23 | 0.80 | −5.5 | −36 | −7 | | |
| M 24 | 0.80 | −6.0 | −62 | −24 | | |
| M 27 | 0.16% NaCl<br>0.012% K | −4.5 | 0 | 0 | +9.0 | |
| M 29 | 0.17% NaCl<br>0.03% K<br>0.007% Ca | −8.1 | −1 | 0 | −2.3 | −0.7<br>−0.7 |
| M 28 | Phenolsulphonephthalein | −4 | −42% Phenolphthalein | | | |

The experiments show that water was absorbed by the cloaca in practically all cases, irrespective of the concentration of the sodium chloride used and whether its concentration was higher or lower than that of the blood. Smaller amounts were absorbed when the chloride concentration was approximately that of the blood; in one case no water or chlorides were absorbed in $3\frac{1}{2}$ hours. In three cases only did an increase of the concentration of the salts occur; when using distilled water without saline, and when using a saline solution of 0.026 per cent concentration there was an increase in NaCl in the cloacal fluid, and when using a fluid containing 0.012 per cent potassium an increase in the amount of potassium was found: using 0.03 per cent potassium, absorption of potassium occurred. In the two experiments where phenolsulphonephthalein was used, absorption of water took place; in one case the coloring matter was excreted from the ureters in less than one-half hour after placing in the cloaca; 42 per cent of the dye used being excreted in $4\frac{1}{2}$ hours; in the other case where a weak solution was used, none was excreted in the urine for one hour and then a small amount was found. In some experiments, the basic radicals Na, K and Ca were estimated and these were found to move with the acid Cl radical. Complete estimations in every case were difficult for the quantities of fluid obtained were small.

The concentration of sodium chloride in the urine collected from the ureters was 0.04 to 0.09 gram per 100 cm.

It is interesting to find that Shoji (2) working with cats and rabbits reports that the bladder epithelium was permeable to water and to NaCl under physiological conditions. He found, however, that with 0.5 per cent NaCl water was absorbed but no movement of NaCl was seen; with 0.25 per cent NaCl and less concentrations, water was absorbed but NaCl increased; with 2 per cent or more, water was given to and NaCl absorbed from the solution. In birds the movement of water and NaCl did not bear a direct relation to the concentration.

## CONCLUSIONS

1. Birds can absorb water and also salts NaCl, KCl, $CaCl_2$ from the cloaca.

2. The amount of water absorbed does not depend solely on the salt concentration of the content of the cloaca.

## BIBLIOGRAPHY

(1) Sharpe: This Journal, 1912, xxxi, 75.
(2) Shoji: Journ. Physiol., 1920, liv, 239.
(3) Meyers: Journ. Lab. Clin. Med., 1920 vi, 17.
(4) Kramer and Tisdale: Journ. Biol. Chem., 1921, xlviii, 233.
(5) Kramer and Tisdale: Journ. Biol. Chem., 1921, xlvi, 339, 467.
(6) Kramer and Tisdale: Journ. Biol. Chem., 1921, xlvii, 475.